4 February 2005

Science

Vol. 307 No. 5710
Pages 629–796 $10

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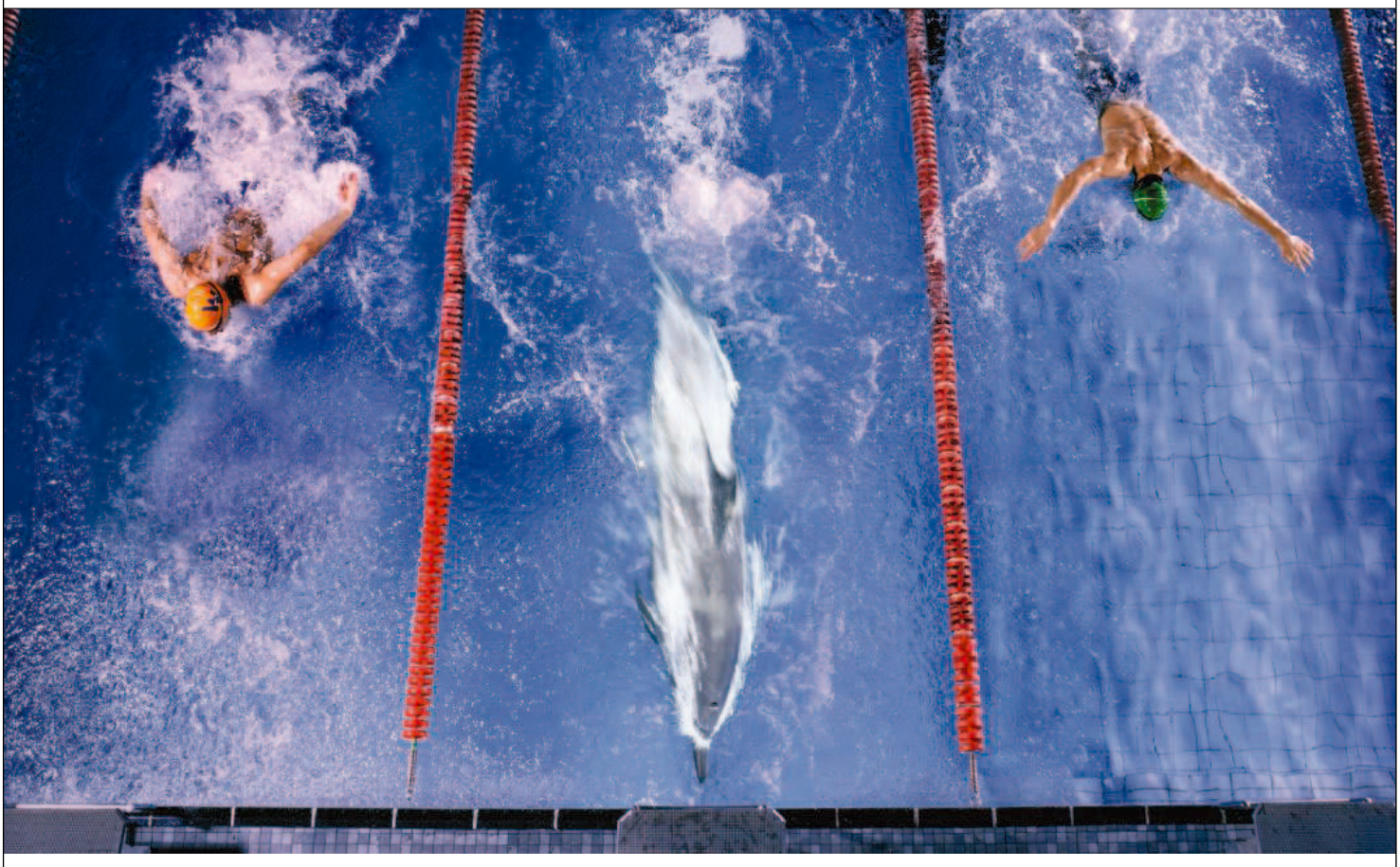

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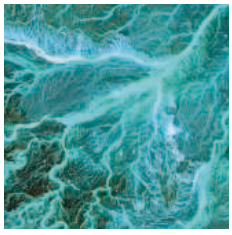
GE imagination at work

Science

Volume 307
4 February 2005
Number 5710



COVER Meandering, rain-swollen wadis combine to form branching networks across the arid landscape of southeastern Jordan. This false-color scene was acquired in May 2001 by the ASTER instrument onboard NASA's Terra satellite. The Gordon Research Conference on Visualization in Science and Education will be held 3 to 8 July 2005 at Queen's College, Oxford, UK. The schedules for the 2005 Gordon Research Conferences begin on page 746. [Image: NASA and USGS]

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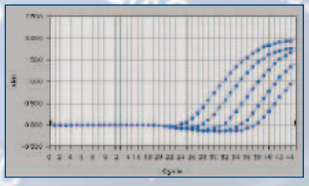
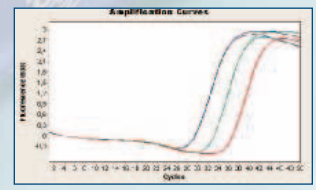
Real-time, multiplex PCR can be so simple — hit all targets in one tube!

Up to 4plex PCR



Detection of t(8;14) translocation sequence in duplex PCR using the QuantiTect Multiplex PCR Kit



Detection of CSBG sequence in triplex PCR using the QuantiTect Multiplex PCR NoROX Kit

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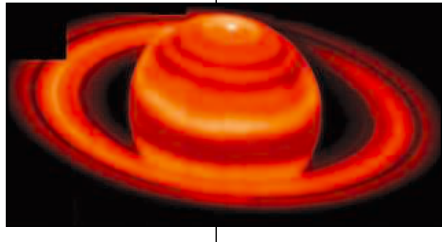

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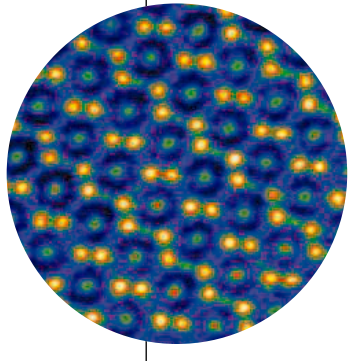

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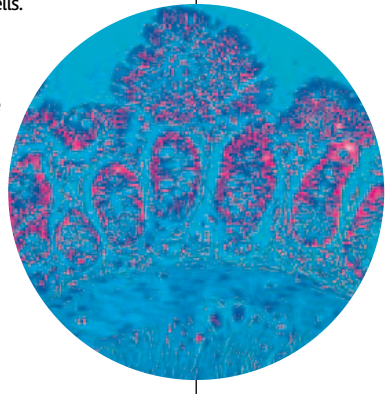

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The Dream Difference
Socially aggressive dreams are more likely to occur during REM than non-REM sleep.

New Trigger for Breast Cancer
Research implicates virus-associated immune system component in the disease.

Extinguished Earth
What would the world look like had there been no forest fires?

Engineering at tribal colleges.

science's next wave www.nextwave.org Career Resources for Young Scientists

Postdoc Network: NIH Multiple-PI Policy May Open Opportunities for Postdocs *B. Benderly*
Whether postdocs benefit from new federal policy will depend on lab and university politics.

MiSciNet: Creating Engineering Programs in Tribal Colleges *E. Francisco*
With help from NSF, tribal colleges will create engineering programs leading to the bachelor's degree.

Canada: Weeding Out the Bugs *A. Fazekas*
Vice president of R&D at a bio-agricultural firm talks about her journey from bench to boardroom.

France: French Postdocs Abroad—Finding Your Way Back Home *E. Pain*
Next Wave speaks to two French postdocs who have left France for the United States and Japan.

Europe: European Science Bytes *Next Wave Staff*
Read the latest funding, training, and job market news from Europe.

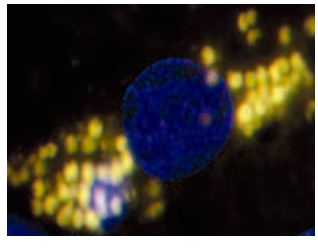
GrantsNet: February 2005 Funding News *Edited by S. Otto*
This is the latest index of research funding, scholarships, fellowships, and internships.

science's sage ke www.sageke.org Science of Aging Knowledge Environment

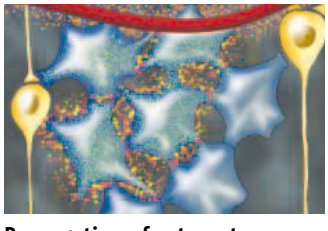
Review: Lipofuscin and Aging—A Matter of Toxic Waste *D. A. Gray and J. Woulfe*
Accumulation of this pigment might cause catastrophic lysosomal and proteasomal inhibition.

News Focus: Ageless No More *M. Leslie*
Once thought immortal, gut bacteria suffer aging's toll.

News Focus: Plugged Up *R. J. Davenport*
Broken pump abets calcium overload after a stroke.



Garbage catastrophe?



Propagation of astrocyte waves.

science's stke www.stke.org Signal Transduction Knowledge Environment

Perspective: Alpha Subunit Position and GABA Receptor Function *D. R. Burt*
Experiments with linked subunits reveal the importance of subunit position to $GABA_A$ receptor function.

Perspective: Reaching Out Beyond the Synapse—Glial Intercellular Waves Coordinate Metabolism *A. Charles*
Synaptic activity stimulates glial calcium waves, which elicit secondary sodium and metabolic waves that may produce lactate as an energy substrate for neurons.

Teaching Resource: Introduction—Overview of Pathways and Networks and GPCR Signaling *R. Iyengar*
These lecture materials introduce general principles and emerging theory in cell signaling research.

Separate individual or institutional subscriptions to these products may be required for full-text access.

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	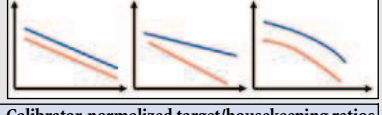		
	Calibrator-normalized target/housekeeping ratios		
40 ng	1.03	1.18	1.41
8 ng	2.21	1.79	1.01
1.6 ng	6.00	4.17	1.17
Mean	3.08	2.38	1.21
S.D.	2.60	1.58	0.22
C.V.	**84.3%**	**66.4%**	**18.0%**

Figure 1: Impact of different PCR efficiency adjustments on accuracy of relative quantification. Total RNA was used for quantitative RT-PCR on the LightCycler System. Sample data were evaluated with the LightCycler Relative Quantification Software, using the efficiency correction functions described above, to generate calibrator-normalized target/housekeeping ratios. The significantly lower Coefficient of Variation (C.V.) demonstrates the greater accuracy made possible by the LightCycler Software's use of efficiency corrections and a non-linear fit function.

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- Obtain sample concentrations from non-linear standard curves to more precisely quantify low-copy genes, which often suffer from non-linear PCR efficiencies (Figure 1).

Shouldn't accurate quantification be the primary goal of gene expression studies? Contact your Roche Applied Science representative and visit ***www.lightcycler-online.com*** today!

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Saturnian Hot Spot

Ground-based infrared observations of Saturn with the Long Wavelength Spectrometer on the Keck I Telescope on Mauna Kea reveal a hot spot in the atmosphere within 3° of the south pole, a warm polar cap, anomalous temperature bands, and oscillations in temperatures in the southern hemisphere that are not correlated with cloud patterns. **Orton and Yanamandra-Fisher** (p. 696) suggest these features are related to radiative forcing and dynamical forcing that are consistent with 15 years of constant solar illumination of the southern hemisphere as Saturn goes through its southern summer solstice.

Two's Company, Three's a Cloud?

It has long been thought that the in situ creation of new (secondary) cloud condensation nuclei arises mainly from the reaction of gas phase sulfuric acid and water, but the rate of particle formation observed in laboratory studies has been too slow (by many orders of magnitude) to account for the number concentrations found in nature. A faster, ternary mechanism that includes ammonia has been postulated on the basis of theoretical factors. **Berndt *et al.*** (p. 698) now report experimental production of particles from a mixture of sulfuric acid and water at concentrations like those naturally found in the atmosphere, with ammonia at concentrations lower than those normally observed. The measured rate is consistent with that required to explain atmosphere number concentrations.

Fungus Monoculture on the Ant Farm

Leaf-cutting ants live in obligate ectosymbiosis with clonal fungi that they rear for food. These symbionts are vertically transferred during colony foundation, but fungus gardens are, in principle, open for horizontal symbiont transmission later on. **Poulsen and Boomsma** (p. 741) show that fungal ectosymbionts prevent competing fungal strains from becoming established by ancient incompatibility mechanisms that have not been lost despite millions of years of domestication and single-strain rearing by ants. These fungal incompatibility compounds travel through the ant gut to make the ant feces incompatible with unrelated strains of symbiont. Thus, the fungi manipulate the symbiosis to their own advantage at the expense of the ants' potential interest in a genetically more diverse agriculture.

The End of the Line

The breaking of the translation symmetry of crystals at their surfaces gives rise to localized surface electronic states, and, in principle, similar effects should be seen at the ends of one-dimensional wires. **Crain and Pierce** (p. 703) present experimental evidence for such electronic states at the ends of one-dimensional gold chains of gold grown on the stepped Si(553) surface. Scanning tunneling microscopy images show markedly different contrast for the end atoms of chains when the bias voltage is reversed, and differential conductance measurements reveal the details of the electronic states of the end atoms that agree well with the results of tight-binding calculations. The formation of end states helps lower the energy of filled states for atoms within the chain.

CREDITS: (TOP TO BOTTOM) POULSEN AND BOOMSMA; CRAIN AND PIERCE

Glimpses into the P/T Boundary

The Permian-Triassic extinction was the most extreme in Earth's history. It has been difficult in part to determine the environmental conditions that may have led to the extinction. **Grice *et al.*** (p. 706, published online 20 January 2005) present a detailed chemical analysis of marine sections obtained by drilling off western Australia and South China. The data suggest that the upper part of the oceans at the time of the extinction were extremely oxygen poor and sulfide rich. **Ward *et al.*** (p. 709, published online 20 January 2005), in contrast, reconstruct a record of the terrestrial vertebrate extinctions in the Karoo Basin, Africa. This area preserves the most detailed vertebrate fossil record from this time, but correlating rocks in different parts of the Basin has been problematic. Using paleomagnetism and carbon isotopes, they show that extinctions were accelerated up to a pulse at the boundary, and that the pattern of appearance of Triassic fauna may imply that some originated even before the final pulse.

Protein Kinase Inhibition Revealed

An important target of the second-messenger cyclic adenosine monophosphate (cAMP) is protein kinase A (PKA). PKA, which regulates processes as diverse as growth, memory, and metabolism, exists as an inactive complex of two catalytic subunits and a regulatory subunit dimer. cAMP binds to the regulatory subunits and facilitates dissociation and activation of the catalytic subunits. **Kim *et al.*** (p. 690) have determined the 2.0 angstrom resolution structure of the PKA catalytic subunit bound to a deletion mutant of the regulatory subunit (RIα). The complex provides a molecular mechanism for inhibition of PKA and suggests how cAMP binding leads to activation.

A Matter of Scale

A striking feature of morphological diversity across animal species is the variability in the relative size, or allometry, of different appendages. Virtually nothing is known of the forces that underlie the evolution of scaling relationships. Using the butterfly species *Bicyclus anynana*, **Frankino *et al.*** (p. 718) tested the roles of developmental constraints and natural selection in determining the size of the wings relative to the body, which as a measure of wing loading has clear functional and ecological importance. Artificial selection experiments on the size of the forewing relative to overall body size resulted in a rapid evolutionary response. In this case, developmental constraints did not limit the evolution of the scaling

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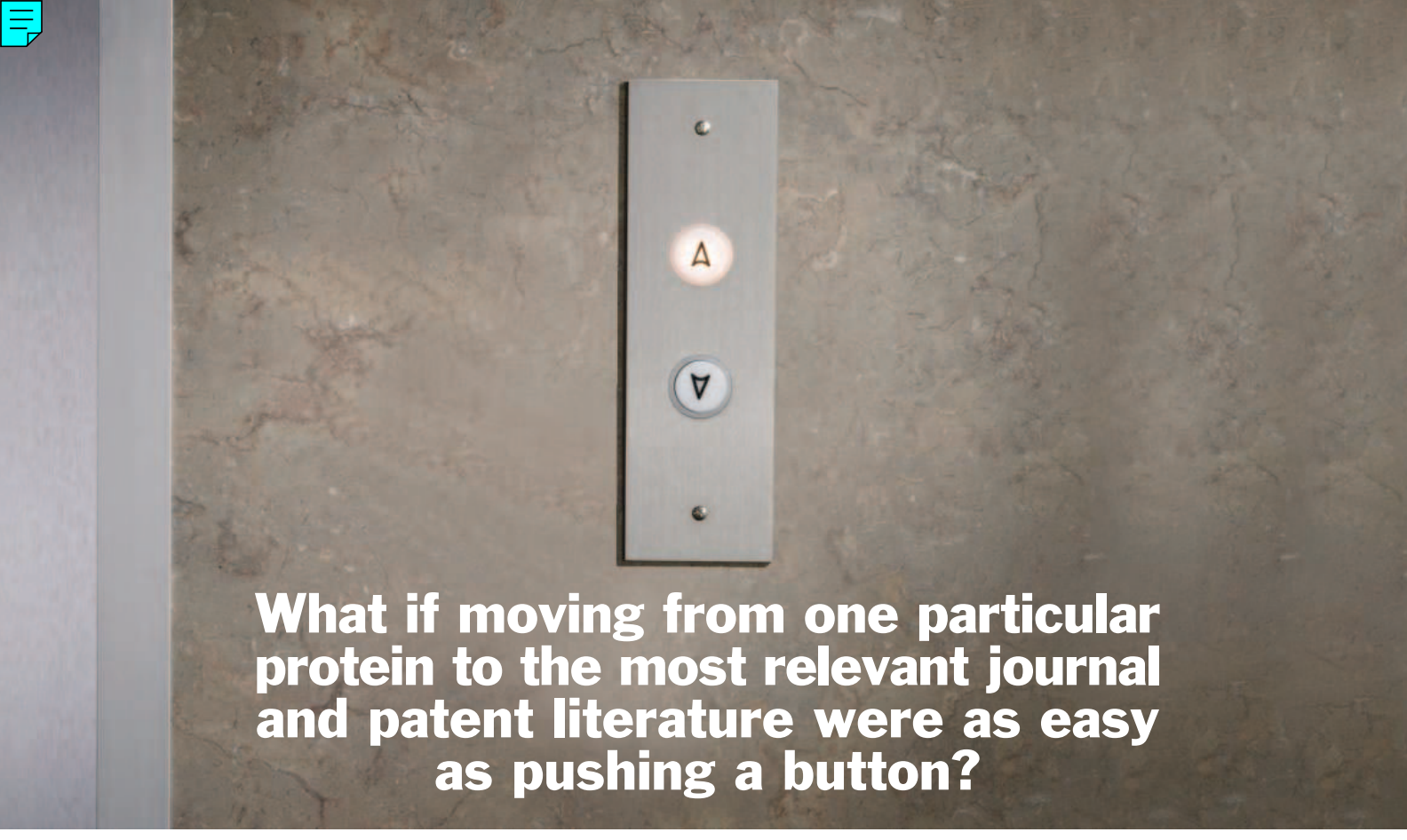

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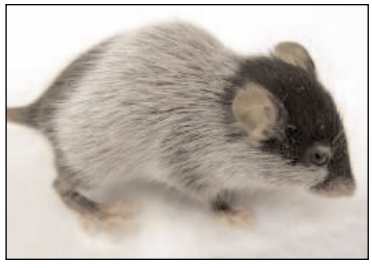
relationship. Instead, it is the pattern of natural selection imposed by the external environment that determine the wing-body size allometry.

Packaging and Power Combining

Mitochondrial DNA (mtDNA) is packaged with proteins into a nucleoid. **Chen *et al.*** (p. 714) show that one of the mtDNA packaging proteins is the Krebs cycle enzyme, aconitase, that the mitochondrion uses to generate metabolic energy. In this second role, aconitase is required for mtDNA maintenance under particular metabolic conditions. This finding provides a direct link between energy generation and mtDNA stability, mitochondrial disease, and aging.

Fade to Gray



Aging brings on many changes in the human body, among them the graying of hair. **Nishimura *et al.*** (p. 720; published online 23 December 2004) found in a mouse model of hair graying that a deficiency of the gene *Bcl-2* caused progressive loss of pigment cells in the bulge of the hair follicle—the hair stem-cell niche. Thus, the physiology of hair graying involves defective self-maintenance of melanocyte stem cells with aging, and may serve as a paradigm for understanding aging mechanisms in other tissues.

Autophagic Arms Race

One defense against intracellular invaders is to enclose them within autophagic vacuoles that then fuse with degradative lysosomes to destroy the pathogen. **Ogawa *et al.*** (p. 727, published online 2 December 2004) show that the invading bacterial pathogen *Shigella* can be recognized and trapped by autophagy. Generally, the pathogen circumvents the autophagic event by secreting an effector protein called IcsB during multiplication within the host cytoplasm; mutant bacteria lacking IcsB are particularly susceptible to autophagic killing. The *Shigella* VirG protein acts as the target that stimulates autophagy, but the IscB protein can camouflage it.

Giving Mice the Nod

The detection of bacteria in the gut by the immune system is regulated, in part, by the Nod proteins, which recognize peptidoglycan motifs from bacteria, and there is a strong association of the inflammatory bowel disorder Crohn's disease with mutations in the *Nod2* gene. Nevertheless, questions remain about the normal physiological role of the Nod proteins in maintaining homeostasis in the gut and how impaired Nod function leads to inflammation. **Maeda *et al.*** (p. 734) observed that Nod mutations in mice, corresponding with those carried by Crohn's disease patients, increased susceptibility to intestinal inflammation caused by the bacterial cell wall precursor muramyl dipeptide. **Kobayashi *et al.*** (p. 731) generated Nod2-deficient mice. Although these animals did not spontaneously develop intestinal inflammation, they were more susceptible to oral infection with the bacterial pathogen *Listeria monocytogenes*. Production of a group of mucosal antimicrobial peptides was particularly diminished in Nod2-deficient animals, which suggests that a similar defect may contribute to inflammatory bowel disease in humans.

Cytokine Production and Kaposi's

When tissues are infected with Kaposi's sarcoma–associated herpesvirus (KSHV), they produce large amounts of proinflammatory cytokines that are linked to disease progression. **McCormick and Ganem** (p. 739) show that a viral protein, kaposin B interacts with mitogen-activated protein kinase–associated protein kinase 2 and enhances the activity of this host cell protein, serving to block the decay of AU-rich messenger RNAs and increase the level of secreted cytokines. This result explains the association of KSHV-related disease and enhanced cytokine production.

CREDIT: NISHIMURA *ET AL.*

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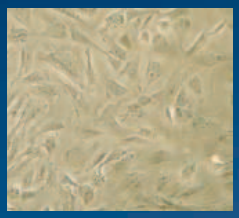

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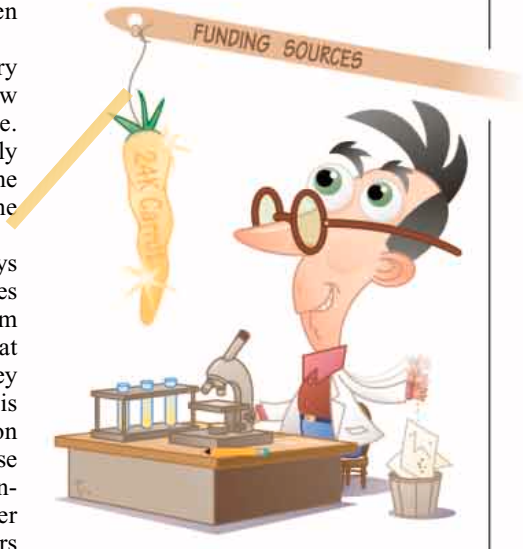
Desirable Scientific Conduct

Many scientists are aware of the subtle influences on their own scientific conduct, but many others are not. Sydney Brenner, the joint winner of the 2002 Nobel Prize for physiology or medicine, delightfully described a slide in which data points were scattered very close to a straight line—but a large mysterious black object lay in one corner. By degrees, the onlooker realizes that the object is a thumb placed over a data point that is far away from the straight line. The thumb covered it up because the result was supposedly anomalous. The test tube was dirty, the animal was sick that day, or something else was amiss. Many other such rationalizations—perhaps they should be called rules of thumb—are produced and the damage done may not serious.

A famous example of selective use of the thumb was provided by the data on which Gregor Mendel based his laws of inheritance. The eminent statistician R. A. Fisher argued that, on grounds of probability, the data were too good to be true. Mendel had presumably started to see (correctly as it turned out) a pattern while he was still doing the critical breeding experiments and then began to drop data that did not fit.



Another form of data selection can lead to serious error. Before the discovery of stratospheric ozone holes in the 1980s, statistical analysis of satellite data threw out the "outliers" on the assumption that such measurements were unreliable. It was only when scientists working at one station in Antarctica repeatedly obtained low values that the processing mistake was discovered and the ozone holes recognized. Treasure your exceptions! The data point lying under the researcher's thumb might be the most interesting result of the whole study.

Social psychologists and sociologists have long been aware of the subtle ways in which bias can creep into research. The behavior of their subjects sometimes results not from the effects of any experimental manipulation, but merely from the attention paid to them by the experimenter. Much evidence suggests that experimenters often obtain the results they expect to obtain, partly because they unwittingly influence the outcome of the experiment.* The expectancy effect is sometimes comparable in size to the effect of the experimental manipulation itself. Many scientists take appropriate steps to avoid this kind of bias. They use "blind" procedures so that the person making the measurements cannot unconsciously bias the result. Analysis is carried out ideally while the researcher remains unaware of the identity of each group. Although many are careful, others are not and do not even recognize the problem.

Suspect findings damage unnecessarily the reputation of scientists for integrity, lending weight to the more bizarre views about the social construction of science. The reality of prejudice or theoretical conformism in scientific work emphasizes that a considerable job of educating many members of the scientific community is still needed. That kind of awareness becomes all the more necessary when issues of funding and promotion are at stake. Some notorious cases have demonstrated just how ferocious can be the pressure from commercial funders to ignore good scientific practice. A well-known example was the shameful treatment at the University of Toronto of Nancy Olivieri, who published data uncongenial to the drug company that had funded her.†

Sources of funding can undoubtedly exert corrupting influences on scientific behavior. The bad cases should be condemned when they are discovered. "Affiliation bias" may, however, be much more subtle, leading research workers to select evidence suiting their own preconceptions. All scientists need to be very careful about how evidence was obtained in the first place. Desirable modes of scientific conduct require considerable self-awareness as well as a reaffirmation of the old virtues of honesty, scepticism, and integrity.

Patrick Bateson

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*R. Rosenthal, D. B. Rubin, *Behav. Brain Sci.* **1**, 377 (1978). †D. G. Nathan, D. Weatherall, *N. Engl. J. Med.* **347**, 1368 (2002).

10.1126/science.1107915

CREDIT: TERRY E. SMITH

edited by Gilbert Chin

EPIDEMIOLOGY

Don't Keep Hedgehogs

Rescuing a hedgehog victim of a road accident and nurturing it back to health can be deeply satisfying. But Riley and Chomel show that the payback from such an exotic pet may not be entirely benign. Hedgehogs harbor a variety of pathogens that are potentially transferable to humans and our livestock. Several species of hedgehogs have been widely introduced into the United States and are kept illegally in some states, in the extraordinary number of 40,000 households. A recent survey shows that they can carry foot-and-mouth disease virus, *Salmonella*, and *Mycobacteria*, as well as dermal fungal infections. Thousands of years ago, domestication brought humans into contact with a range of new pathogens; the current vogue for exotic pets and food animals will do likewise, namely, monkeypox and plague in prairie dogs and SARS in civets. —CA

European (top) and African pygmy (left) hedgehogs.

Emerg. Infect. Dis. **11**, 1 (2005).

CELL BIOLOGY

Popeye's Ribosomes

Ribosomes are the central component of the protein synthesis machinery, and the efficient manufacture of ribosomes is crucial. These machines contain roughly 60 protein and RNA parts. The assembly of these parts occurs in the nucleus and involves importing proteins from the cytosol and placing them onto ribosomal RNA (rRNA); subsequently, the assembled small and large ribosomal subunits are exported to the cytosol.

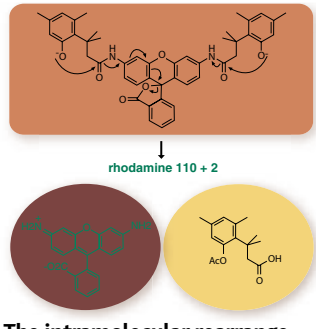
Two groups describe an unanticipated link in this chain of events—iron-sulfur (Fe-S) cluster biosynthesis. In a screen for ribosomal export mutants, Yarunin *et al.* found that genes implicated in Fe-S cluster biosynthesis in the cytosol are needed for ribosomal export. In particular, the protein Rli1 requires a Fe-S cluster to promote rRNA processing and small ribosomal subunit export. Kispal *et al.* also implicate Rli1 in the export of ribosomal subunits from the nucleus. In addition, they present evidence that this explains why Fe-S cluster biogenesis is an essential function and thus why mitochondria (where Fe-S cluster biogenesis originates) are essential. — SMH

EMBO J. 10.1038/sj.emboj.7600540; 10.1038/sj.emboj.7600541 (2005).

CHEMISTRY

Unlocking Fluorescence

The power of fluorescent probes can be enhanced by controlling how and when the probe becomes excitable. Chandran *et al.* describe an approach for masking fluorescence from a xanthene fluorophore until it is cleaved by esterases within a cell. They coupled rhodamine 110 to two *o*-hydroxycinnamic acid derivatives via amide linkages. In their acetylated form, these side chains force the rhodamine core to adopt a nonfluorescing lactonized configuration. Ester cleavage causes the side chains to form a hydrocoumarin, which is favored by steric interaction of the methyl groups, and liberates the fluorescent acid form of rhodamine 110. The authors followed the uptake of the latent fluorophore into HeLa cells, where they observed strong fluorescence from the cytosol and lysosomes but not from the nucleus. — PDS



The intramolecular rearrangements that liberate rhodamine (left).

J. Am. Chem. Soc. 10.1021/ja043736v (2005).

GEOLOGY

Protection Against Erosion

River and stream erosion rates and the resulting river profiles are becoming more widely recognized as depending on a complicated and incompletely documented suite of factors. For instance, some streams and rivers appear to be eroding their bedrock at the geologically extreme and unsustainable rates of several centimeters per year; hence, other processes must be contributing to bedrock dynamics. Over a 7-year period, Stock *et al.* monitored several rivers in Taiwan and in the Pacific Northwest of the United States. These rapidly eroding rivers had all been historically scoured of sediment. This history and the authors' measurements imply that long-term stream erosion, at least in areas with weak bedrock, is influenced more by the ability of rivers to entrain a thin covering of sediment, which reduces wear, than specific bedrock properties. In areas of high slope, debris flows, which periodically scour streams and rivers and thus allow rapid downcutting, may be the most critical factor. — BH

Geol. Soc. Am. Bull. **117**, 174 (2005).

BEHAVIOR

Less Editing, Less Depression

A number of recent studies have fueled a sense of optimism that the fuzzy link between genes and behavior might be firmed up and made explicit, an especially challenging task given the likelihood that the contributions of individual genes (and distinct mutations) to behavior might be only a few percent of the total mix of predisposition, motivation, and environment. Biogenic amines are, of course, front and center in any consideration of mood and affect, and genes encoding various aspects of serotonin function in neurons (synthesis, transport, and receptors) have already been targeted as prime candidates for dysfunction in depression.

Englander *et al.* have used a pair of mice strains to examine the interaction of

serotonin receptors, stressful situations, and a selective serotonin reuptake inhibitor (SSRI). They report that, in comparison to C57BL/6 mice, BALB/c animals have lower serotonin levels (due to a polymorphism in tryptophan hydroxylase-2) and are generally easier to stress (via a behavioral despair task). Furthermore, the type 2C serotonin receptor in BALB/c mice undergoes less editing of its pre-messenger RNA, and this yields, in compensatory fashion, receptors that are more sensitive to serotonin. Administering the despair task or the SSRI (the antidepressant fluoxetine) bumps up the extent of RNA editing and presumably titrates downward the responsiveness of postsynaptic neurons to released serotonin. The unexpected finding is that this change in editing due to drug or stress is not seen if both are given together, suggesting that the molecular response may be influenced by the state of the subject and blocked by antidepressants. — GJC

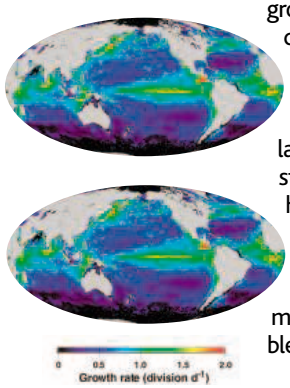
J. Neurosci. **25**, 648 (2005).

EARTH SCIENCE

Getting a Fix on Fixation

Marine net primary production (NPP) is a measure of how much atmospheric carbon is fixed via photosynthesis by organisms in the ocean. Until now, only direct field sampling has yielded accurate estimates of NPP, which has severely limited attempts to obtain detailed global estimates of its distribution and magnitude. Measuring NPP from space has failed to provide convincing values, because two essential parameters, phytoplankton carbon biomass and a term related to the physiological status of the organisms, are not directly quantifiable remotely.

Behrenfeld *et al.* start with satellite measurements of the chlorophyll content of upper ocean waters and the backscattering of certain wavelengths of light (which they use to estimate phytoplankton carbon biomass), and then estimate phytoplankton growth rates and calculate NPP. They can do this by taking advantage of laboratory studies that have shown that the ratio of chlorophyll to carbon biomass is a calculable function of changes in light, nutrients, and temperature. This work brings nearer the prospect of producing a more accurate picture of global marine NPP over space and time. — HJS



Phytoplankton growth rates during the boreal summer (top) and winter (bottom).

Global Biogeochem. Cycles **19**, 10.1029/2004GB002299 (2005).

CREDITS: BEHRENFELD *ET AL.*, *GLOBAL BIOGEOCHEM. CYCLES* **19**, 10.1029/2004GB002299 (2005).

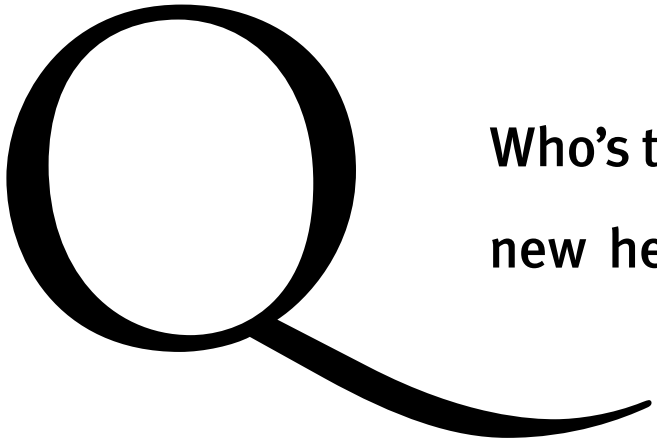
HIGHLIGHTED IN *SCIENCE*'S SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

Science's stke www.stke.org

Balancing Axons and Dendrites

Neurons are polarized cells with axons (signal output) and dendrites (signal input). Not only are these functionally distinct parts of the cell, but they differ in morphology too. Jiang *et al.* report that when glycogen synthase kinase 3β (GSK-3β) activity was increased by transfection of isolated embryonic hippocampal neurons with a constitutively active mutant, the number of cells that formed an axon decreased, and when GSK-3β activity was inhibited, the number of cells producing multiple axons increased even though the overall number of neurites did not change. They identified the phosphatidylinositol 3-kinase (PI3K) pathway as a stimulator of GSK-3β phosphorylation, which results in an inhibition of GSK-3β. Activation of the PI3K pathway by expression of the kinase Akt or inactivation of the phosphatase PTEN produced multiaxon neurons. Yoshimura *et al.* show that GSK-3β phosphorylates collapsin response mediator protein 2 (CRMP-2), which is known to contribute to axon formation. Treatment of neurons with neurotrophin 3 (NT-3) or brain-derived neurotrophic factor (BDNF) stimulated axon growth and decreased CRMP-2 phosphorylation. Furthermore, the stimulation in axon length was blocked if CRMP-2 abundance was decreased. Thus, a pathway involving PI3K regulates the activity of GSK-3β and the phosphorylation of the microtubule assembly regulatory protein CRMP-2, and hence controls axon formation and growth in neurons. —NG

Cell **120**, 123; 137 (2005).



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AAAS member Dr. R. Douglas Fields, senior scientist, developmental neuroscience

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Photo: Dr. R. Douglas Fields

Douglas Fields' son Dylan takes a break on the way up

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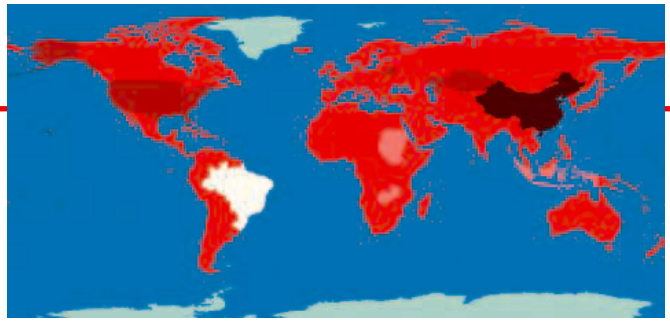
Information for Contributors

See pages 135 and 136 of the 7 January 2005 issue or access www.sciencemag.org/feature/contribinfo/home.shtml

NetWatch

edited by Mitch Leslie



DATABASE

Planet Earth Checkup

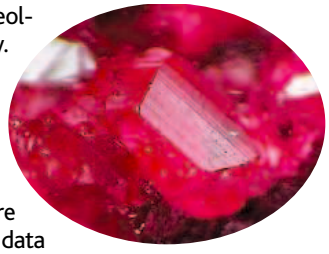
Whether you're interested in the number of threatened plant species in various countries, changes in glacier mass, airborne lead levels, or the use of ozone-depleting compounds such as methyl bromide, check out the Global Data Portal from the U.N. Environment Programme. The site lets you download data on more than 450 economic and ecological variables or render them as a map, graph, or table. The chart above, for example, depicts the change in forest cover for different countries between 1990 and 2000, with Brazil showing the biggest loss and China recording gains. The figures collected here provide the underpinnings for the U.N.'s Global Environment Outlook, an occassional report on the biosphere's condition, and other summaries.

geodata.grid.unep.ch

IMAGES

Mineral Mother Lode

These glittering crystals of roselite (below) owe their crimson hue to cobalt, which constitutes about 10% of their weight. Find out much more about roselite—from its chemical composition to the origin of its name—at Webmineral, an exhaustive database maintained by Houston, Texas–based geology consultant David Barthelmy. Since NetWatch's last visit (*Science*, 11 June 1999, p. 1731), this compendium of 4300 minerals has added photos for more than half the entries and Java applets that let you study each crystal's structure from multiple angles. You'll also find data such as the minerals' hardness rating, x-ray diffraction values, classification according to the Strunz and Dana systems, and other tidbits. For example, roselite isn't named for its ruddy color, but for Gustav Rose, a 19th century German mineralogist.



webmineral.com

LINKS

Math From the Ground Up

Researchers who specialize in the foundations of mathematics delve into deep topics such as set theory and proof theory to ensure that math rests on solid philosophical ground. For a comprehensive take on the subject, visit this site from Alexander Sakharov, a computer scientist and Web developer in Boston. Sakharov has arranged more than 50 links into chapters to create a virtual textbook, with writings from mathematicians, computer scientists, and other experts. The contributions—which include books, articles, and entries in references such as the *Stanford Encyclopedia of Philosophy*—probe topics like mathematical logic and axioms.

sakharov.net/foundation.html

RESOURCES

Taking the Sting Out of Bumblebee Taxonomy

They may be as close to cuddly as insects can get, but bumblebees give taxonomists headaches because different species inhabiting the same area often look alike. For help navigating the group's treacherous taxonomy, make a beeline for this site from entomologist Paul Williams of the Natural History Museum in London. His checklist of world bumblebees—the first published since 1923—attempts to tidy up the nomenclatural mess. You can find out which types of bees live in North America and Europe or search the site by bioregions, such as eastern Asia. The checklists discuss valid and invalid names for each kind of bee. To aid identification, the entries also include photos of the male bees' genitalia, a key feature for differentiating species. Above, a male *Bombus asiaticus* prowls for a mate.

www.nhm.ac.uk/entomology/bombus/index.html

DATABASE

All Together Now

To deduce a protein's function, researchers need to know everything from its structure and location in the cell to what molecules it interacts with. But this information resides in disparate databases that often use different terminology, and compiling it "can be a painful experience," says computer scientist Golan Yona of Cornell University. So Yona and his colleagues crafted Biozon, a database that merges the holdings of more than a dozen molecular biology collections, including SwissProt, KEGG, PDB, and BodyMap. The site lets you run searches that span different data types, such as finding 3D structures for all proteins that interact with the protein BRCA1, which is implicated in some breast cancers. The ability to compare results from different databases side by side also makes it easier to spot discrepancies.

biozon.org

Send site suggestions to netwatch@aaas.org. Archive: www.sciencemag.org/netwatch

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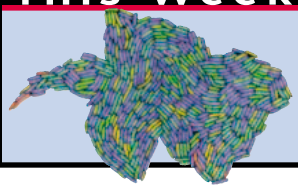
This Week



PAGE 656
Aging bacteria

657
Corals after the tsunami

BIOMEDICINE

Move Provokes Bruising Fight Over U.K. Biomedical Institute

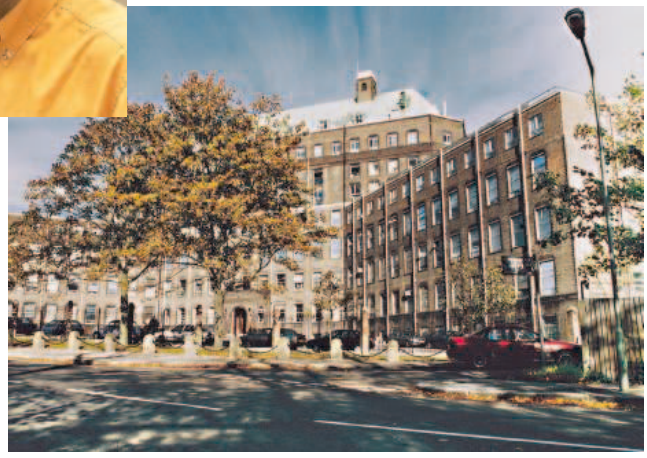
CAMBRIDGE, U.K.—For more than a year, researchers at a world-class biomedical institution in Britain have been battling to stop what they see as a clumsy and destructive attempt to overhaul their community. Next week, Parliament will give its view of their appeal to block a relocation from the suburbs to the center of London, possibly followed by a clear decision from their top governing board, the Medical Research Council (MRC). Both sides say this fight may leave bruises that will affect biomedical research in the United Kingdom for years.

Leading scientists at the National Institute for Medical Research (NIMR)—the largest U.K. biomedical unit, with a direct government budget of $62 million—took an angry protest to the halls of Parliament in December. In the parliamentary inquiry that began that same month, they blasted an MRC plan to move their entire facility from its perch on a green ridge northwest of London to a university site in the city. The staff's main concern, according to NIMR immunologist Anne O'Garra and others who spoke with *Science*, is that the advantages of the present spot in Mill Hill will be lost—including a secure animal facility and an unparalleled 19-hectare campus—with no commensurate gain.

MRC's preferred scheme for "renewing" NIMR, says neuroscientist Colin Blakemore, MRC's chief executive, is to sell the entire NIMR estate. Director John Skehel has announced that he will retire in 2006, but the scientific staff would be kept intact. The cash from the sale, as Blakemore explained in the 1 December hearing before the House of Commons Science and Technology Committee, would help pay for new facilities in central London. The final details have not been set, but an MRC task force has singled out Kings College and University College as candidate sites. It's not clear whether the government would own the new city buildings or pay a university to maintain them. Yet Blakemore concedes that moving NIMR from Mill Hill may "cost more than it will save."

Blakemore cites several reasons for wanting to move NIMR despite the cost. He told the science committee that government recommendations dating back to 1996 and earlier have urged that the institute be brought closer to a university. And in a telephone interview, he spoke of MRC's long-held concern that NIMR is too isolated from academic and clinical life. It must change to survive, he and others argue.

A July 2004 MRC task force on the renovation plan, which included two NIMR scientific leaders, concluded that the institute's "long-term success" will depend on its ability to do "translational research": adapting basic biology to medical uses. NIMR supports more than a dozen fields of research and is known for its excellence in infections and immunity, developmental biology, neuroscience, and structural biology, among others. But it has no clinical center and no degree-granting function. The task force found that NIMR could best make a "cultural shift" from its focus on laboratory work to clinical practice "through physical proximity to a teaching hospital." And it found that encouraging crosstalk among different research groups "would best be achieved through colocation with a university" with "the widest possible range of disciplines."



Auction? Colin Blakemore, the U.K. biomedical research funding director, defends a plan to sell off the 19-hectare Mill Hill facility and move the staff into the city center.

The two NIMR scientists who sat on the task force signed off on this report—Steven Gamblin, head of the protein structure group, and Robin Lovell-Badge, head of developmental genetics. But both now disagree with MRC's interpretation. They say they support the broad conclusions but not Blakemore's views on relocating to London. As they tell it, the consensus wanted a move to the city if this proved *better* than staying at Mill Hill. But in their view, MRC has not really compared the options in detail and is ignoring the conditional clause. Lovell-Badge also told the parliamentary committee that in a phone call Blakemore had pressured him to drop his resistance, but that he refused. Blakemore acknowledged that there was a conversation but denied that he intended any threat.

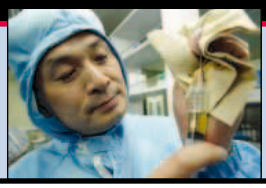
Gamblin, Lovell-Badge, O'Garra, and others argue that NIMR already enjoys many of the good things that the move to London might bring, such as interdisciplinary collaborations and partnerships with clinics. They offer piles of documents as proof. But they concede the obvious: that NIMR is not physically near a teaching hospital or university. On the other hand, they say, it's doubtful any London university can provide a secure, spacious animal facility like theirs. Gamblin and Lovell-Badge voiced a widely held suspicion that the university-dominated MRC wants to sell Mill Hill to have more flexibility in the budget for academic projects—and to subject NIMR staffers to the rigors of university life.

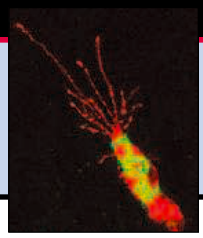
Blakemore says he has heard the rumors, but they are "completely unfounded." There is "no hidden agenda," he adds: "If the MRC had wanted to close down the NIMR, they would have done it" long ago and not have "spent two and a half years reviewing it."

Parliament's science committee will offer its view of the controversy in a report to be issued on 8 February. And Blakemore says MRC hopes to issue its own decision on 10 February.

—ELIOT MARSHALL

CREDITS (TOP TO BOTTOM): BARKER EVANS PHOTOGRAPHY; NIMR



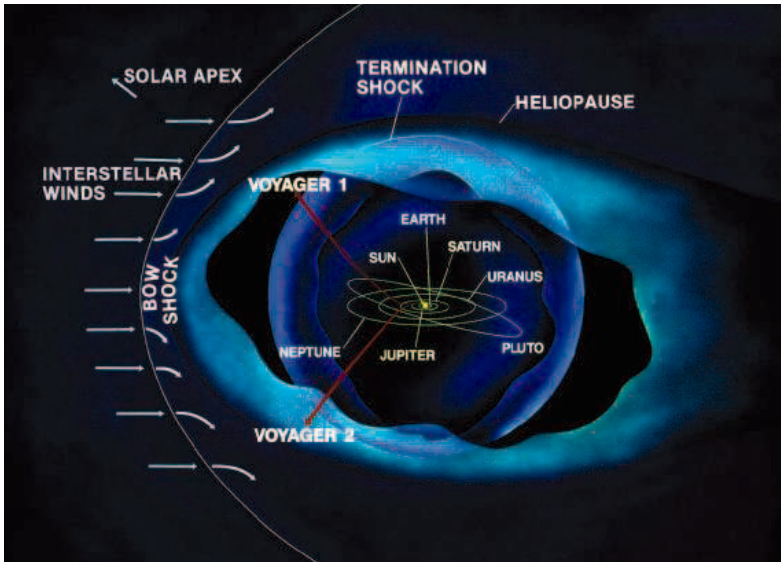


SPACE SCIENCE

NASA Probe to Examine Edge of Solar System

Despite its current budget troubles, NASA last week laid out plans to launch a mission to explore the edge of the solar system. The $134 million probe, slated for a 2008 launch, will also serve President George W. Bush's exploration vision by examining galactic cosmic rays that pose hazards to humans traveling beyond Earth's orbit.

Southwest Research Institute of San Antonio, Texas, will lead the mission, called the Interstellar Boundary Explorer (IBEX). Chosen from more than three dozen proposals, IBEX is part of NASA's Small Explorer effort designed to put relatively low-cost probes into orbit more quickly than the agency's usual space science missions. "This is an exciting and breakthrough experiment for NASA to sponsor," says Ghassem Asrar, the agency's deputy science chief. No other mission has attempted to chart the heliopause—the bubblelike transition zone where the solar wind breaks down—in such detail. IBEX won't actually make the long and expensive trip beyond Pluto. Instead, by flying in an Earth orbit beyond the magnetosphere, the spacecraft's two neutral-atom imagers can detect particles that will enable physicists to map the boundary between the solar system and deep space.



Outer limits. Earth-orbiting IBEX satellite will map the region where particles from the sun meet the tenuous currents of interstellar space.

Although IBEX appears set to proceed, another Small Explorer mission—the Nuclear Spectroscopic Telescope Array, designed to detect black holes—has been delayed on technical grounds. The project, by a team at the California Institute of Technology in Pasadena, will not get a green light until at least next year, NASA officials say, because the design needs further study. And tight funding, combined with the recent reorganization of NASA's science effort, temporarily threw planetary researchers for a loop last week. On 24 January, Curt Niebur, discipline scientist for the outer planets research program, announced that $5 million for data analysis had been "redirected" and that "the future of the program is far from secure." But 3 days later, acting director of NASA's solar system division Andrew Dantzler wrote researchers that "the funding has not been cut" but simply moved to another area "for purely administrative reasons." Dantzler blamed the mix-up on a "miscommunication" within NASA.

–ANDREW LAWLER

AIDS TREATMENT

A Step Toward Cheaper Anti-HIV Therapy

The U.S. Food and Drug Administration (FDA) approved three generic anti-HIV drugs last week, a decision that finally allows U.S. government–sponsored programs to offer these cheaper pills to infected people who live in poor countries.

The Bush Administration insisted last year that the so-called President's Emergency Plan for AIDS Relief—which plans to spend $15 billion over 5 years—could only use drugs approved by FDA. Howls of protests followed, as many AIDS advocates and clinicians worried that this would rule out use of cheap treatments now popular in many poor countries. The Administration promised to process completed applications from generic manufacturers within 2 to 6 weeks.

Aspen Pharmacare of South Africa began the application process in September (*Science*, 8 October 2004, p. 213). On 25 January, 12 days after FDA received Aspen's completed application, it "tentatively" approved three of its drugs. (The tentative designation means that, for patent reasons, the drugs can be sold for use only in poor countries.) "I'm very pleased," says Anthony Fauci, head of the National Institute of Allergy and Infectious Diseases. "This is what we were hoping would happen." The approved Aspen drugs include a pill that combines AZT and 3TC, and, separately, nevirapine. "I hope that a lot of other companies follow suit, and we can get the ball rolling with these generics," says Fauci.

Ellen 't Hoen, a lawyer based in Paris who heads the Campaign for Access to Essential Medicines run by Médecins Sans Frontières, argues, however, that FDA approval is unnecessary. The World Health Organization (WHO) has already "prequalified" many generic AIDS drugs that are in an even easier-to-use formulation—three pills mixed into one fixed dose—and U.S. insistence on additional approval by FDA "creates confusion and has wasted time and money," says 't Hoen. "To celebrate this FDA approval as a major breakthrough is presenting a false picture."

According to a WHO report released on 26 January, anti-HIV drugs currently reach only 700,000 of the nearly 6 million poor people in the world who most urgently need treatment.

–JON COHEN

CREDIT: COURTESY NASA/JPL-CALTECH

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QUANTUM COMPUTING

Safer Coin Tosses Point to Better Way For Enemies to Swap Messages

Alice and Bob are finally splitsville. After years of sending encrypted messages to each other, they're getting a divorce. They've moved away from each other and only communicate electronically, but this creates a problem. "They want to decide who's going to keep the dog, so they toss a coin," says Alipasha Vaziri, a physicist at the National Institute of Standards and Technology in Gaithersburg, Maryland. How to do a fair coin flip over a telephone wire?

Physicists have now shown how, using quantum computers. In an upcoming issue of *Physical Review Letters*, Vaziri and his colleagues describe an experiment in which Alice and Bob perform a fair coin flip quantum-mechanically; if one party tries to cheat, the deception is quickly revealed, something that scientists don't know how to guarantee with classical computers.

At odds. Is it possible to make a fair flip with a hidden coin, untrustworthy opponents, and no referee? Quantum physics says yes.

The fair electronic coin flip is what cryptographers term a "post–Cold War" protocol. In standard cryptography, two parties who trust each other attempt to sneak a message by an untrustworthy opponent; here, two parties who don't trust each other try to ensure that the other isn't cheating. So a good coin flip protocol should ensure that Alice, who wants to keep the dog, can't cheat Bob.

With classical computing, coin flips are tricky. There's no provably secure way for Alice to flip the coin and have Bob call the toss—and ensure that one party or the other can't cheat when determining the winner. (They could sidestep the problem by having a trusted third party do the coin toss for them. However, the only two-party classical algorithms rely on mathematical constructs that nobody is certain are tamper-proof.)

With quantum computing, though, the picture changes. Vaziri, along with physicist Anton Zeilinger of the University of Vienna and other physicists, exploited the quantum-mechanical property of "entanglement" to ensure that neither side can cheat. In their setup, Alice has a pair of photons (created by an argon-ion laser shot at a barium-borate crystal) that are entangled: Measure one and you instantly affect the other's properties.

Alice tosses the "coin" by forcing one photon's angular momentum to take one of four possible states, two of which represent "heads" and two of which represent "tails." This changes the state of the other entangled photon, which is sent to Bob. Bob measures his photon, but because quantum ambiguity makes different pairs of the four states look the same, he's unable to determine whether Alice picked heads or tails. He calls the flip—tells Alice heads or tails—and then Alice reveals which of the four states she picked, allowing Bob to verify instantly whether he won or lost the toss.

Bob can't cheat, because he doesn't know the outcome of the flip before transmitting his guess to Alice. And it's a subtler point, but Alice can't cheat because the signature of the coin flip is inscribed in Bob's photon; if she tries to lie, then this deception will likely show up as "noise," nonsensical data when Bob interprets his measurements of the photon. Although there's only a certain probability of catching Alice each time she cheats, it's probabilistically guaranteed that she'll be caught if the ownership of the dog is determined by 1000 coin flips.

"The big problem in the paper was to come up with cheating algorithms," says Zeilinger, who adds that Alice would have a very hard time gaming the system. "We feel that our procedure is very safe."

The experiment is important "because coin tossing is a task in bigger protocols, such as multiparty communications," says Andris Ambainis, a physicist at the University of Waterloo in Canada. "When you have sufficiently strong coin tossing, you multiply what you can do securely" over communications lines, whether the parties trust each other or not, he says. And given the torrid lives of Alice and Bob, cryptographers will likely stay busy for many years to come. —CHARLES SEIFE

CREDIT: BRAND X PICTURES/GETTY IMAGES

ScienceScope

Florida Rejects Chiropractic Program

Florida's Board of Governors has killed a proposal to set up a chiropractic school at Florida State University (FSU). The board's 10–3 vote last week against the proposal, developed by FSU after the state legislature endorsed a $9-million-a-year spending plan, caps months of protests by faculty members who viewed the school as a threat to the university's scientific reputation (*Science*, 14 January, p. 194).

"There's no way that the program can now be resurrected," says FSU Provost Lawrence Abele. "I'm glad we don't have to drag the faculty through a long and protracted discussion" over the scientific merits of chiropractics, he says. Before voting, board members said they expected that a new private college near Daytona Beach would produce more chiropractors than Florida needs.

The board's decision comes as a "big relief," says Raymond Bellamy, director for surgery at FSU's Tallahassee campus, who helped organize faculty opposition. Besides hurting FSU's standing as a research university, he says, the proposed school "would have been a horrible waste of taxpayer money." —YUDHIJIT BHATTACHARJEE

Europe, U.S. Differ on Mercury

The European Commission is proposing an international initiative to phase out mercury production and prevent surpluses from flooding the world market. The new strategy, released this week, would permanently shutter a major mine in Spain and ban all exports by the European Union, the world's largest supplier of mercury, starting in 2011. The proposal will be discussed later this month at a meeting of the United Nations Environment Programme in Nairobi, Kenya.

In contrast, the United States is mulling the idea of countries forming voluntary partnerships to assess the problem of mercury. But environmentalists say that approach won't reduce either demand or supply. —ERIK STOKSTAD

Italy Pulls Out of Global Fund

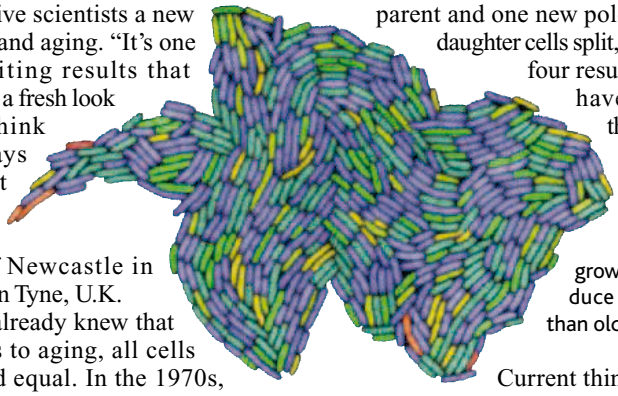
Italy has decided to withhold a promised contribution of $130 million this year to the Global Fund, a partnership of private and public agencies devoted to fighting AIDS, tuberculosis, and malaria. The cut is part of a $325 million reduction in government assistance to nonprofits this year due to a tight economic climate. Mariangela Bavicchi, a spokesperson for the fund, calls the decision an example of "regrettable behavior at the international level."

—MARTA PATERLINI

MICROBIOLOGY

Immortality Dies as Bacteria Show Their Age

"I don't want to achieve immortality through my work," Woody Allen once said. "I want to achieve it by not dying." Although that's unlikely for Allen and other higher organisms, many biologists believed that immortality was possible for microbes. Now, however, a new study suggests that bacteria get old, a finding that may give scientists a new tool to understand aging. "It's one of those exciting results that makes you take a fresh look at what you think you know," says gerontologist Thomas Kirkwood of the University of Newcastle in Newcastle upon Tyne, U.K.



Inevitable decline. Young *E. coli* (blue) grow faster and produce more offspring than old *E. coli* (red).

Biologists already knew that when it comes to aging, all cells are not created equal. In the 1970s, Kirkwood offered the disposable-soma theory: Cells of the body, or soma, can deteriorate, but the germ line cells have to take better care of themselves because they give rise to sperm and eggs. Simple organisms such as budding yeast engage in a subtler division of labor; aging yeast parents invest their freshest components in their buds. But biologists believed that immortality was possible for microbes that divide into identical-looking daughter cells.

To test that assumption, microbiologist Eric Stewart of INSERM in Paris tracked the fate of individual *Escherichia coli* cells. The rod-shaped bacterium divides in half to form two identical-looking daughter cells, which contain one old end, or pole, inherited from the parent and one new pole. When those daughter cells split, only two of the four resulting cells will have poles from the original cell. Current thinking assumed that all four cells were the same.

The INSERM team tracked the growth of single cells and their descendants on a specially designed microscope slide, taking images every 2 to 4 minutes for up to 6 hours. Ultimately, they tracked 94 colonies, consisting of more than 35,000 individual cells. By comparing 7953 pairs of sister cells, the researchers discovered that cells that inherited the older pole of the parent grew 2.2% more slowly than those that inherited a younger pole. The bigger the difference in age, the bigger the difference in growth rate, they report in the February *PLoS Biology*—a result the team attributes to "decreased metabolic efficiency." The results mean that even "an apparently symmetrically dividing organism is subject to aging" and "make it unlikely that natural selection produced an immortal organism," Stewart says.

Leonard Guarente, who studies aging at the Massachusetts Institute of Technology, agrees, saying that the results "put the onus of proof on anyone who claims that cells can be immortal."

Biogerontologist George Martin of the University of Washington, Seattle, calls the results "conceptually very important" but cautions that the cells that slow and stop reproducing may just be taking a break to repair themselves. If further study shows that the older cells are actually dying off, it would "make biogerontologists take seriously the notion that there's aging in bacteria." And if *E. coli* does get old, researchers could use it to study "how aging occurs and how it's regulated," Guarente adds, allowing them to get "right to the molecular heart of the matter."

—DAN FERBER

U.K. UNIVERSITIES

Cash-Short Schools Aim to Raise Fees, Recruit Foreign Students

CAMBRIDGE, U.K.—Even Britain's top research universities say they're broke. Although they receive regular government subsidies (see p. 668), the law limits what they can charge students for tuition. (The rate is about $2170 per year at present.) In addition, compared to U.S. institutions, they get only modest gifts from alumni and philanthropies. The result is "chronic underfunding," says a strategic plan released last week by the University of Oxford.* The problem is growing worse, according to the document put out by Oxford's vice chancellor John Ford on 24 January, and "radical" changes are needed, including higher student fees.

Currently, Oxford's income is running about $38 million per year below expenditures, according to Ford's strategic planning paper, and "nearly all of the university's core activities lose money." The Oxford University Press helps reduce the deficit by transferring at least $23 million per year to the university. To help slow the leakage, says Oxford spokesperson Ruth Collier, the university will take advantage of a new law next year that will allow variable tuition charges for U.K. students up to about $5662 a year. The university also hopes to recruit more foreign students, who pay many times the domestic rate. Collier says the motivation is not to raise funds, because that would bring in an additional $4.7 million per year—"a tiny proportion" of the annual revenue. Rather, the goal is to make the university more competitive in the world market.

Other U.K. universities are doing likewise, including the nation's largest, the University of Manchester, which disclosed similar plans to raise tuition fees and recruit foreigners in January.

Although Oxford counts itself among the world's top five research universities, the paper notes, its "fundraising efforts … pale in comparison with those of the leading U.S. universities." Oxford raised about $110 million overall in 2002–03, the paper points out, while in that period Harvard and Stanford raised about $495 million and $472 million, respectively. Only 5% of Oxford's alumni make annual donations, compared to 40% to 60% of rivals' alumni.

Oxford's student body has been expanding at 1.5% per year, while the academic staff has "remained static," according to the report, and between 1979 and 1999, the ratio of students to teachers "deteriorated from 9:5 to 13:2." The plan aims to reverse that trend. It also calls for boosting the fraction of foreign students from 7% to 12%. But it won't be easy.

Oxford and other top universities are already under pressure from the government to increase the fraction of students they accept from U.K. state schools. Student and political leaders, meanwhile, are lobbying against university plans that might make admissions easier for foreign students. National Union of Students President Kat Fletcher decried an approach, as she told the *Guardian*, that treats "international students as simply pound signs that will solve the funding shortfall."

The Oxford plan has gone out to students, faculty, and others for comment until mid-March; after that, it will be considered for a final decision.

—ELIOT MARSHALL

*www.ox.ac.uk/gazette/2004-5/supps/strategy.pdf

CREDIT: ERIC STEWART AND STEFANIE TIMMERMANN/INSERM

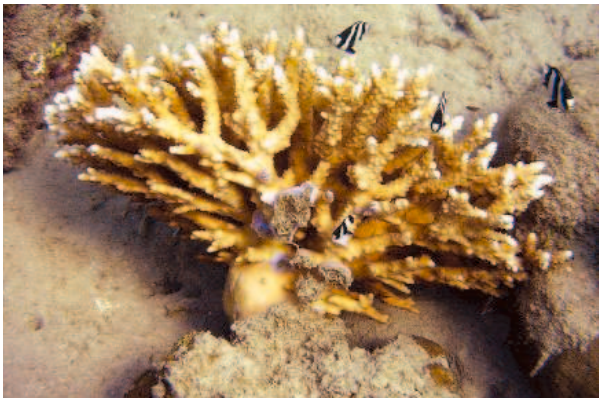
SOUTH ASIA TSUNAMI

Powerful Tsunami's Impact on Coral Reefs Was Hit and Miss

Early surveys suggest that coral reefs around the Indian Ocean survived December's tsunami in better shape than many had feared. In the sites where researchers have looked, "only a few areas were severely damaged, and the rest should recover rapidly in the next 5 to 10 years," says Clive Wilkinson, a marine scientist with the Australian Institute of Marine Science in Cape Ferguson. In some places, divers are already helping that recovery with restoration efforts.

In the immediate aftermath of the 26 December tsunami, Wilkinson and others feared the worst. The wave's awesome power, as well as sediment, pollutants, and debris washed onto the reefs when the wave retreated, posed major threats, says Russell Brainard, a U.S. National Oceanic and Atmospheric Administration oceanographer based in Honolulu. If eroded mud and silt buried a reef, they could destroy the corals.

Off the coast of Thailand, however, many reefs were spared. In January, volunteers and academic and government marine scientists took to the water for a first look. They evaluated 175 sites in the Andaman Sea along the west coast of Thailand, rating each according to the degree of impact. Half or more of the coral was missing from 13% of the sites, says Thamasak Yeemin, a marine scientist from Thailand's Ramkhamhaeng University in Bangkok. About 40%, though, seemed untouched.



Uprooted. The tsunami left some reefs untouched, but in many places—as shown above—it knocked down corals of all shapes and sizes.

CREDIT: KEVIN MOSES/AP PHOTO

Also last month in Thailand, Sakanan Plathong, a marine biologist at Prince of Songkla University in Had Yai, and 60 assistants armed with cameras spent 3 weeks combing a smaller area, the Similan Islands, one of the country's best dive spots. "In general most Similan islands that are dive sites are still in good condition," says Plathong. Only about 15% of the area's coral was severely damaged.

David Obura of CORDIO East Africa—a collaborative coral research program in the Indian Ocean—in Mombasa, Kenya, has similarly good news about the African coast. "We were generally surprised at the [small] and very patchy damage to the coral reef communities," he says.

Although turbid water prevented local government and academic divers from looking at six of the 10 sites in the Seychelles selected for a preliminary assessment, the survey indicated that only 13% of the coral colonies were damaged.

The reefs that were affected suffered different levels of damage, some repairable and some not. Corals were toppled over, sometimes covered with sand and rubble. In some places, meter-high sea fans were pummeled and knocked off their perches. Several reefs were littered with debris—logs, beach beds, towels, palm trees, boat engines, and beach umbrellas. These wave-driven objects "become like bulldozers," says Brainard. "They severely erode the coral habitat."

After the tsunami, Plathong realized he had to act fast to save any damaged corals. He brought a brigade of 136 volunteer divers to some of the worst places in the Similan Islands. The divers worked to right corals and were able to salvage those that hadn't slid beyond reach down the sloping sea floor. They propped up sea fans—a temporary fix until they could return with marine cement. They also removed debris, although heavy objects had to be left behind. The repair efforts benefited in one way from the tsunami's power: The wave was so strong that potentially lethal silt and mud washed far out to sea in many areas.

Yet there were places "where the reefs were just planed off and stripped to bare rock," says Wilkinson. In another part of Thailand, three of four reefs surveyed were decimated. In some places, divers measured 5 millimeters of sediment on top of the corals. The reefs off the Tamil Nadu cost in Southeast India also appear to be severely damaged, as was coral off the Andaman and Nicobar Islands. "It will take some time before we can build a proper picture of the ecological ramifications of this disaster," says Wilkinson.

–ELIZABETH PENNISI

ScienceScope

NIH Bans Industry Consulting

Responding to an uproar last year over industry consulting by staff, the National Institutes of Health this week announced a ban on all such interactions by NIH intramural scientists. Many staffers will also have to sell their stock in biotech and drug companies.

NIH took a hard look at its consulting policies, which were loosened in 1995, after a December 2003 report in the *Los Angeles Times* suggested improprieties and Congress investigated. Last year, NIH Director Elias Zerhouni proposed new limits, including a 1-year ban on all consulting. This week he followed through by releasing an interim regulation that will implement the ban until further notice. The policy does not restrict NIH employees from receiving some payments for teaching, writing, or editing.

Meanwhile, the inspector general of the Department of Health and Human Services is investigating the conduct of Trey Sunderland, an Alzheimer's disease researcher at the National Institute of Mental Health. Sunderland is said to have received more than $500,000 from Pfizer since 1999 without first asking for approval or reporting the income.

Sunderland has accepted a job at Albert Einstein College of Medicine in New York City but has not left NIH yet. His lawyer, Robert Muse of Washington, D.C., declined to comment, but he has told NIH that its "indifference" was why Sutherland failed to file the necessary paperwork.

–JOCELYN KAISER

Call for Global Biodiversity Agency

PARIS—Researchers from around the world have endorsed a call by French President Jacques Chirac for a new international organization for biodiversity research—akin to the Intergovernmental Panel on Climate Change (IPCC)—that would sift through the science and identify priorities for nations. An IPCC-like agency could provide the field with a stronger, unified voice, says Michael Loreau of Pierre and Marie Curie University in Paris, who chaired the scientific committee of a UNESCO meeting held here last week.

The 1500 scientists and politicians attending the meeting had little to celebrate. The loss of species continues apace, and a 2002 goal of achieving a "significant reduction" in the rate of biodiversity loss by 2010 appears doomed. Besides more science, "we also need action—now," says Loreau.

–MARTIN ENSERINK

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TAIWAN

University Spending Plan Triggers Heated Debate

TOKYO—Taiwan has adopted a $1.6 billion plan to strengthen its research universities, reigniting a debate over how much of the money should go to a handful of leading institutions. A decision rests with a new cabinet now being assembled.

On 20 January legislators voted to allocate $315 million a year for 5 years to refurbish university facilities and boost faculty salaries. Under rules set out by the previous cabinet, most of the money would go to schools with 25,000 students or more, with the goal of turning them into world-class universities. To be eligible, the schools would also need to take steps to become private, not-for-profit institutions—part of a broader campaign to streamline the government that also includes reducing the 100-plus universities on the island. Taiwan currently has only two institutions of that size—National Taiwan University in Taipei and National Cheng Kung University in Tainan. The rest of the money would be spent on research centers affiliated with a dozen or so universities with active research programs.

Greener outlook? National Taiwan University would likely benefit from the new spending plan.

The Ministry of Education has published statistics showing that Taiwan is not keeping pace with its neighbors in supporting its leading universities. Per capita spending at the flagship National Taiwan University was one-12th the amount at the University of Tokyo, it noted, and one-eighth that of the National University of Singapore. The survey "confirmed the shocking disparity among the institutions," says Chen Teh-hua, director of the ministry's Department of Higher Education.

The money, expected to begin flowing in June, will be parceled out in a competitive process. But the real fight will be over whether the criteria that will govern the competition will be revised following a December setback to the ruling Democratic Progress Party in parliamentary elections that has forced a reshuffling of President Chen Shui-bian's cabinet.

On one side are those who say Taiwan's best chance of moving up the global academic ladder is by concentrating resources. "Higher education is a competitive sport. If you don't give resources to your best, you won't be able to compete on the world stage," says Frank Shu, an astronomer who in 2002 left the University of California, Berkeley, to become president of National Tsinghua University in Hsinchu. The new funding scheme has increased speculation that Tsinghua may merge with its neighbor, National Chiao Tung University, creating an institution large enough to occupy the first tier.

Chiang Wei-ling, vice president of National Central University, says he and his colleagues at smaller schools recognize the need to give greater funding to a few top universities. "But it shouldn't be a binary, win-or-lose situation," says Chiang. "You have to have a pyramid, with some concentration at the top but enough incentive so other universities don't get discouraged." A 2003 report from a committee of top academics and government leaders recommended a 60–40 split.

But the proper balance isn't the only issue. The chair of that committee, former National Central University president C. H. Liu, says that privatizing higher education "is extremely controversial and is opposed by both government and opposition legislators." Liu and others are hoping that the cabinet shakeup will give the government a chance to rethink the entire policy.

–DENNIS NORMILE

UNITED KINGDOM

Proposed Law Targets Animal-Rights Activists

A new law could send animal-rights activists in Britain to jail for up to 5 years if they cross the line between peaceful protest and harassment or intimidation. The new rules, introduced in Parliament on 31 January as an amendment to a larger anticrime bill, would specifically outlaw campaigns that target businesses that provide supplies or services to research organizations. It would also make it illegal to protest "outside someone's home in such a way that causes harassment, alarm, or distress to residents," according to a government statement.

Barbara Davies of RDS (formerly the Research Defense Society) in London, a lobby group that defends animal research, said the law could provide important support. "There have been amendments to laws on harassment and intimidation before, and we thought that would work. But it's getting worse," she says. Key advantages of the new law, she says, are that it would make it easier for authorities to charge the organizers of campaigns and increase protection for companies that work with organizations that do animal research. Last summer, work stopped at a new research lab in Oxford after construction company shareholders received threatening letters from activists (*Science*, 23 July 2004, p. 463). And in recent months, animal-rights activists have been charged with dozens of attacks—including desecration of a family grave—against a guinea pig farm near Birmingham that supplies research labs.

Out of bounds? A new law would impose harsh penalties for activists who cause economic harm to researchers or companies.

But some animal-rights activists are worried that the measures go too far. The law could potentially target peaceful protests and boycotts, says Andrew Tyler of Animal Aid in Tonbridge, Kent, which has organized demonstrations against the Oxford lab. The prohibition against demonstrations in front of residences could allow police to shut down protests at university research facilities that happen to be near residential buildings, he worries. "Like fishermen, if you cast a wide net, you catch many nontarget species," he says. Parliament is expected to debate the measure this spring.

–GRETCHEN VOGEL

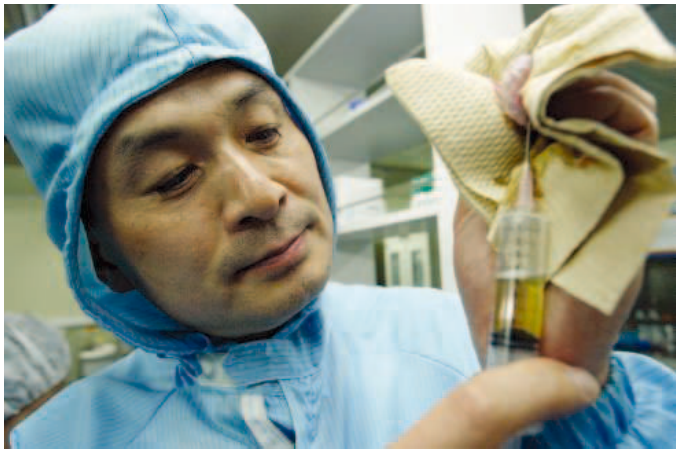
CREDITS (TOP TO BOTTOM): COURTESY NATIONAL TAIWAN UNIVERSITY PRESS; SHAC

Less encumbered by societal restrictions on embryonic stem cells, scientists in the developing countries of Asia are giving Western researchers a run for their money

Asia Jockeys for Stem Cell Lead

Veterinarian Woo Suk Hwang and gynecologist Shin Yong Moon leapt from obscurity to scientific stardom last February when they isolated embryonic stem (ES) cells from cloned human cells, a world first and a key step toward therapeutic, or research, cloning.

Coming from a region that rarely produces scientific headlines, the announcement by the Seoul National University (SNU) pair stunned researchers around the world. But it was no fluke. Hwang has a long track record of successful animal cloning. Moon is South Korea's leading expert in assisted reproductive technology. The duo were able to draw on the expertise of a dozen co-authors at six institutions. And when Western scientists got their first peek into the SNU lab, they were astounded to see state-of-the-art facilities—and an enviable supply of egg donors.



Spotlight. Woo Suk Hwang (above) and Shin Young Moon grabbed acclaim for South Korea with their breakthrough work with ES cells.

Largely below the radar screen, the emerging economies of South Korea, Singapore, Taiwan, and China are fast becoming major centers for human ES cell research. Like their colleagues in the advanced scientific powers—including Japan, the United States, and many European countries—researchers in the developing countries of Asia are racing to learn how to transform ES cells into human tissues and organs, which could lead to treatments for conditions that are now intractable, such as diabetes, Parkinson's disease, and spinal cord injuries.

But there is one big difference: Unlike their colleagues in the United States and much of Europe, Asian scientists have the full support of their governments. Because obtaining ES cells involves the destruction of very early stage embryos, many Western governments have placed heavy restrictions on the work. But across Asia, there is little of the conflict with prevailing religious and ethical beliefs that has Western countries hesitating (see sidebar, p. 664). Governments are ramping up funding for both basic and applied stem cell work, setting up new institutes, programs, and grant schemes, and providing incentives for private companies to join the effort. Giving these efforts a further boost, the region also has legions of lab workers willing to log long hours, and increasing numbers of expatriate scientists are returning home to work in the flourishing environment.

With all these advantages, Asia's scientists believe that they can be fully competitive in, and perhaps even lead, the race to harness stem cells. "Asia has never dominated [any field in] cutting-edge biology," says Chunhua "Robert" Zhao, director of the National Center for Stem Cell Research in Beijing. "This could be our chance."

Stem cell researcher George Q. Daley of Harvard Medical School in Boston agrees: "I firmly believe they have an advantage." Although recent state funding initiatives in California and Wisconsin (see sidebar, p. 662) should ease some of the constraints hobbling ES cell research in the United States, says Daley, such efforts are no substitute for federal support, which is still restricted.

Asia does face challenges, however. These countries are still building their scientific infrastructures, and many institutions must make do with older equipment. For some groups, geographical isolation and lingering language barriers hinder participation in conferences and complicate scientific publishing. In China, a lack of coordination and a culture of secrecy among scientists hamper progress. Perhaps most pressing, says South Korea's Hwang, the entire region suffers from a dearth of experienced senior scientists to run the new programs.

A series of firsts

Asian scientists have been at the forefront of research on cloning and stem cells since its inception. At China's Shandong University, embryologist Tong Dizhou produced the world's first cloned vertebrate, an Asian carp, in 1963. He went on to create the first interspecies clone in 1973, by inserting European carp DNA into an Asian carp egg. But Tong's work remained almost unknown outside China.

Two decades later, in 1994, Ariff Bongso, an in vitro fertilization (IVF) expert at the National University of Singapore, reported the first isolation of human ES cells in the journal *Human Reproduction*. But Bongso was unable to keep the cells growing, so the work attracted little publicity. That changed when two U.S. groups—one led by James Thomson of the University of Wisconsin, Madison, and another by John Gearhart of the Johns Hopkins University School of Medicine in Baltimore, Maryland—almost simultaneously solved the problem of maintaining stable lines of ES cells in 1998 by growing them on "feeder" layers of mouse fibroblast cells. Bongso and colleagues from Monash University in Melbourne, Australia, and Hebrew University in Jerusalem caught up, creating their own stable human ES cell lines in 2000.

Singapore

Singapore was quick to realize the scientific and commercial payoffs of stem cell research. "Given its huge potential, stem cell research has been identified as one of Singapore's niche areas," explains Hwai Loong Kong, executive director of the Biomedical Research Council, a part of Singapore's Agency for Science, Technology, and Research (A*STAR). ES cells became a cornerstone of Singapore's

CREDIT: KIM KYUNG-HOON/REUTERS

State of the art. With a 25-person research team, Singapore's ES Cell International is racing to create insulin-producing ES cells to treat diabetes.

$2 billion National Biomedical Science Strategy, announced in June 2000 (*Science*, 30 August 2002, p. 1470).

Kong says A*STAR is spending about $7.3 million per year to support stem cell research, using both embryonic and adult lines, at the country's national labs and through grants to university researchers. But that is only part of the story. Academic groups also get funding from their universities and the Ministry of Education. The amount can't be pinned down, but the National University of Singapore reports that about a dozen groups are working on stem cells. Additional money is coming through venture capital support for start-up companies working to commercialize stem cell therapies and from foreign funders attracted by Singapore's welcoming climate for ES cell research.

Among academics, Bongso continues to set the pace. In September 2002, he and his Australian and Israeli colleagues reported the first propagation of human ES cells without using mouse feeder layers—a key advance because the lines grown on mouse cells probably cannot be used for clinical applications, given concerns about nonhuman pathogens. Bongso and his colleagues have turned over their cell lines and intellectual property to ES Cell International for commercialization. ES Cell owns six of the 22 human ES cell lines currently listed on the U.S. National Institutes of Health's (NIH's) Stem Cell Registry and has supplied more than 140 ES cell lines to researchers around the world, second only to the Wisconsin Alumni Research Foundation.

In March 2002, ES Cell recruited Alan Coleman, former research director of PPL Therapeutics in Edinburgh, U.K., and a member of the team that cloned Dolly the sheep, to head its 25-person research team. The company is banking on its ability to turn stem cells into insulin-producing cells that could be transplanted into patients with diabetes. Robert Klupacs, ES Cell International's CEO, says it hopes to start human clinical trials in 2006. Ronald McKay, a stem cell researcher at NIH, says ES Cell International is definitely one of the teams to watch, as is Singapore as a whole. Although the company has yet to turn a profit, concedes Klupacs, it has been able to support its $6.1-million-a-year research program with grants from Singapore, Australia, and private investors.

The U.S.-based Juvenile Diabetes Research Foundation (JDRF) is also supporting stem cell research in Singapore. It provided a $600,000 grant to Bernat Soria of the University Miguel Hernandez de Elche in Alicante, Spain, to set up a lab in Singapore in 2002 to continue work he was prevented from doing in his native country. In February 2000, Soria reported that his group had differentiated mouse ES cells into insulin-producing cells that had alleviated diabetes symptoms in mice. He has been extending that work to humans in his Singapore lab. Although the Spanish government has since relaxed its restrictions, Soria plans to keep a lab in Singapore. "The Asia-Pacific is playing a very important role in this research," he says.

JDRF is also putting up half the cost of a $3 million fund—the other half is coming from A*STAR—to support other Singapore-based stem cell researchers working in a number of fields, as part of a new, competitively reviewed grant scheme. The founda-

Asia's Stem Cell Firsts

1963 First cloned vertebrate (Asian carp)
Tong Dizhou
Institute of Oceanology, Chinese Academy of Sciences, Qingdao, Shandong Province, China
Science Bulletin (Chinese)

1973 First interspecies clone (European carp DNA into Asian carp egg)
Tong Dizhou
Institute of Oceanology, Chinese Academy of Sciences, Qingdao, Shandong Province, China
Acta Zoologica Sinica

1994 First isolation of human ES cells
Ariff Bongso
National University of Singapore
Human Reproduction

2002 First propagation of human ES cells without use of mouse feeder layers
Ariff Bongso
National University of Singapore (plus colleagues from Monash University in Melbourne, Australia, and Hebrew University in Jerusalem)
Nature Biotechnology

2003 First isolation of embryonic stem (ES) cells from cloned human cells
Woo Suk Hwang and Shin Yong Moon
Seoul National University
Science

CREDITS (TOP TO BOTTOM): ES CELL INTERNATIONAL; D. NORMILE/*SCIENCE*

U.S. States Offer Asia Stiff Competition

Proposition 71, the $3 billion initiative designed to catapult California into position as the world leader in research involving human embryonic stem (ES) cells, is having a seismic effect across the United States. A few states—notably Wisconsin and New Jersey—are trying to become counterweights to California. Others are proposing more modest measures to make their states more attractive to stem cell researchers. Many legislators are trying to float initiatives despite substantial obstacles, such as big budget deficits. But if they don't take action, "states that have made significant investments in biomedical research"—Maryland and Massachusetts, to name two—"are genuinely concerned they are going to lose intellectual capital and resources," says Daniel Perry of the Coalition for the Advancement of Medical Research in Washington, D.C.

Wisconsin—where the first human ES cell line was derived in 1998—is moving decisively. The state is poised for a massive new investment of $750 million in stem cell and other biomedical research over the next few years, including more than $500 million in new facilities and research support for scientists at the University of Wisconsin, Madison. Post–Proposition 71, a planned $375 million public-private research institute, the Wisconsin Institute for Discovery, has gained impetus.

In New Jersey, acting Governor Richard Codey is pursuing a regional approach. He has proposed allocating $150 million from unspent bond income to construct the New Jersey Institute for Stem Cell Research, a joint project of Rutgers University and the University of Medicine and Dentistry of New Jersey. Codey wants a ballot referendum next November to raise $230 million to bankroll research grants over the next 10 years.

In Illinois, members of the state Senate failed narrowly in November to pass a bill that would have allowed state funding for ES cell and nuclear transfer research. Now state Comptroller Daniel Hynes has designed a California copycat initiative: a statewide referendum in 2006 on a billion-dollar bond initiative. The Illinois Regenerative Medicine Institute would be created from the sale of $100 million in bonds per year for 10 years—repaid through a 6% tax on cosmetic plastic surgery.

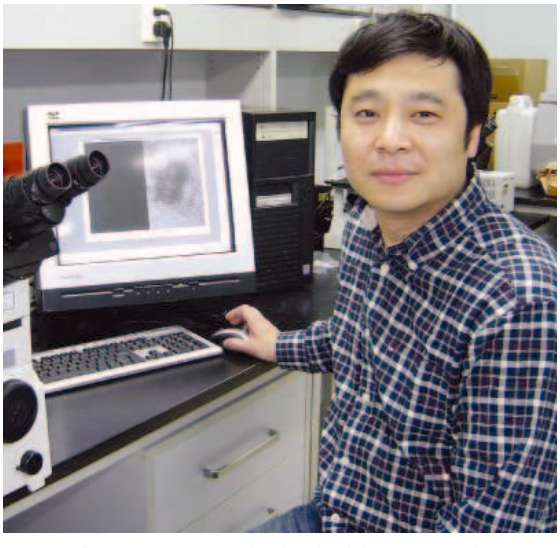
States' rights. New Jersey's Richard Codey is one of several governors trying to lure stem cell research to his state.

tion is investing in Singapore, says Chief Scientific Officer Robert Goldstein, "because there is excellent science, a good environment, and really strong support for work that can't be done in [the public sector in] the U.S.," including deriving and working with new stem cell lines.

China

Although numbers are hard to verify, China may be home to the largest stem cell program in Asia. The government does not release statistics, but Pei Xuetao of the National High Technology Research and Development Program's stem cell division estimates that China has "about 300 to 400" Ph.D.s working on all types of stem cells in more than 30 scientific teams across the country. Perhaps 80 of them work with embryonic cells, a proportion that is growing.

As opposed to Singapore's coordinated national plan, China has a host of overlapping initiatives from the central government, cities and provinces, private enterprise, and even semiprivate venture capital funds created by government agencies and the military. Pei pegs the total 5-year research budget at "more than" $24 million. (Dollars go further there than in the West, given China's vastly lower labor and material costs and its allocation of almost 100% of funding to research, with little overhead.)



Coming home. Deng Hongkui left his lab in New York for new digs in Beijing's Peking University.

Perhaps more important than funding levels, says Xiangzhong "Jerry" Yang of the University of Connecticut, Storrs, is that China has one of the most supportive environments for embryo research anywhere in the world. Issued in December 2003, Chinese regulations are quite "liberal," says Yang. There is a strict ban on human cloning for reproductive purposes. But for research cloning, the guidelines include little more than a requirement that scientists comply "with the principle of informed consent and informed choice" when obtaining embryos from IVF clinics or fetal tissue from aborted fetuses. They also include a directive for institutions to monitor compliance. Wang Yu, the Ministry of Science and Technology's vice director of rural and social development, says the guidelines are "aimed at pushing forward our country's stem cell and therapeutic cloning research."

The competition among groups and the government's reluctance to reveal information make it difficult to judge China's progress. But there is one sign of success: the growing number of foreign-trained Chinese scientists who are leaving comfortable positions in Europe and the United States to work in their native land. Sheng Hui Zhen, an ES cell researcher at Shanghai Second Medical University, says that "most major teams" in the field now have U.S.- or Europe-trained scientists in senior positions.

Sheng is a prominent example. She spent 11 years at NIH before relocating to Shanghai in 1999, where she leads a 50-person team, funded mostly by the city. The group is attempting to create functioning human ES cells by inserting the nucleus of adult human skin cells into rabbit eggs from which the nuclear DNA has been extracted. Sheng reported initial success in August 2003 in *Cell Research*, a peer-reviewed journal backed by the Chinese Academy of Sciences, but so far, no other lab has reported duplicating her work.

The popular press described the work as a "cross-species clone," sparking intense ethical debate in the West. But Sheng dismisses talk of chimeric animals, noting that the only rabbit DNA in the cells is mitochondrial. Her goal, she says, is to design an alternative to human eggs for use in therapeutic cloning. She suspects that when such work becomes feasible, the procurement of eggs, which are

CREDITS (TOP TO BOTTOM): MIKE DERER/ASSOCIATED PRESS; YAN SHEN

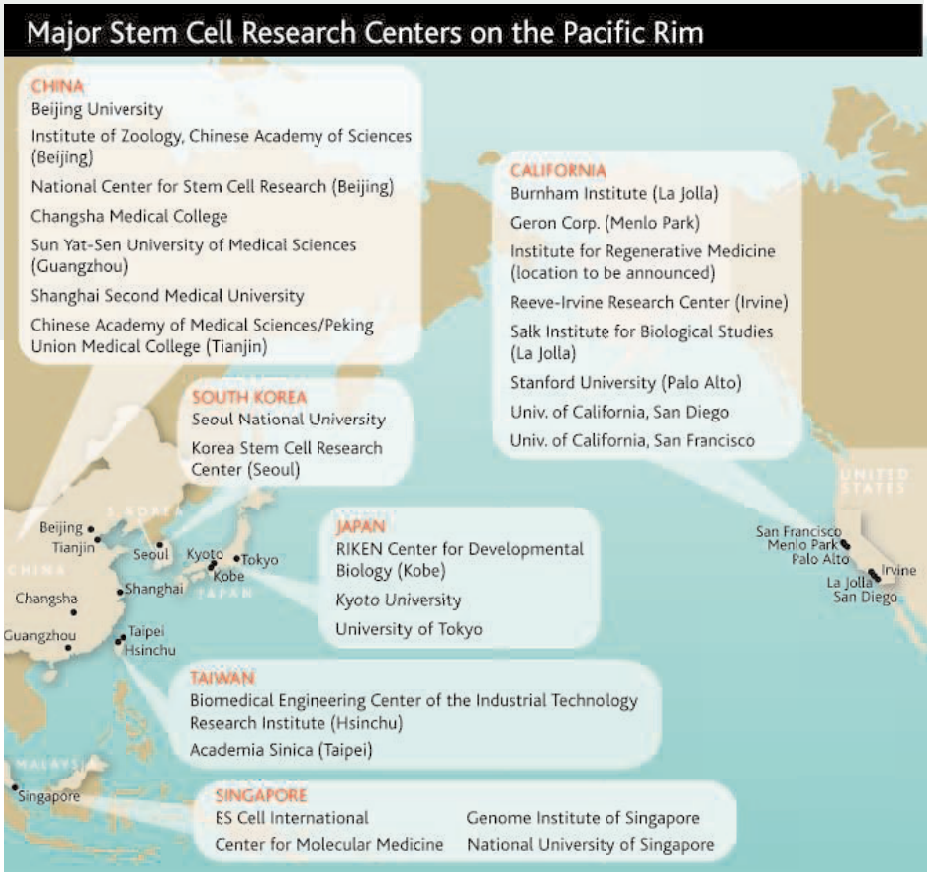
Other states are eyeing various strategies to beef up their stem cell capacities. In Maryland, legislators are readying a proposal that would use tobacco-settlement money to open up $25 million annually for stem cell research starting in fiscal year 2007. Florida is poised to become a major player now that the California-based Scripps Research Institute plans to open its first branch in Palm Beach County. And a private group, Cures for Florida, is campaigning for a $1-billion-plus state ballot initiative for ES cell research.

Legislators in Massachusetts are chafing to get into the stem cell game, but because of the state's large Catholic population, recent pro-research measures have been quashed by the legislature. But Democrats, who are angling for the support of Republican Governor Mitt Romney, have vowed this year to push legislation to promote stem cell research through measures such as tax incentives. And in New York earlier this month, three legislators proposed a 10-year, $1 billion bond initiative that would finance the New York Stem Cell Research Institute.

On the flip side, a number of states are attempting to close the door on research with human ES cells. Nebraska, South Dakota, and Louisiana have forbidden such research. Laws prohibiting nuclear transfer (therapeutic cloning) have been passed in Michigan, Arkansas, Iowa, North Dakota, and South Dakota. Missouri is contemplating one, although scientists are warning that the state will pay a price if it adopts such a ban. The Stowers Institute for Medical Research in Kansas City, a major contributor to the biological lifeblood of the state, has said it "would be forced" to build a planned second facility outside Missouri if the measure passes.

Perry sees the state initiatives as evidence that the center of gravity in research may be shifting away from the federal government. "After generations in which a single NIH" ruled the biomedical research roost, "it's almost like the breakup of the Roman Empire."

–CONSTANCE HOLDEN



difficult and expensive to obtain, may be the weak link. "My Chinese lab does not have everything my NIH lab had," says Sheng. "But here I can work on this important problem, and there I couldn't."

Some Chinese scientists have received backing for research that astonishes their former Western colleagues. Trained in Minnesota, Zhao of the National Center for Stem Cell Research in Beijing is working on stem cells from the bone marrow of aborted fetuses—work that cannot be done with federal funding in the United States and that many states have banned outright. "We have the freedom to look at these problems from many angles," he says.

South Korea and Taiwan

To date in South Korea, the private sector has taken the lead in stem cell research. Three of the four groups that have established ES cell lines are at private IVF clinics, and for their breakthrough work, SNU's Hwang and Moon relied on a culturing technique developed at one of them. Figures for private sector spending are not officially tallied, and Hyun Soo Yoon, director of research at Seoul's MizMedi Hospital, which has a team of 18 scientists and technicians working full-time on stem cell research, also declined to disclose his group's budget.

Now the South Korean government wants to capitalize on the advances made by Hwang and Moon. At Hwang's home university in Seoul, the government is spending $50 million over 5 years to set up the Bio-MAX Institute; its goal is to foster interdisciplinary research in the life sciences, with a major focus on stem cells. Hwang will be moving his lab to Bio-MAX. Meanwhile, Moon continues to direct activities at the Korean Stem Cell Research Center. Established in 2002, the center has an annual budget of $7.5 million to support 30 researchers. And last year, the government put $5 million into a new competitive grant scheme for research related to therapeutic cloning, stem cells, and xenotransplantation. Funding could rise to $25 million per year by 2008.

In Taiwan, the government-affiliated Industrial Technology Research Institute (ITRI) is trying to nurture the island's biotechnology industry by developing stem cell expertise. ITRI researchers were the first in Taiwan to start working with human ES cells, in 2001. They have an 18-person group working to derive their own mouse-feeder-free cell lines and to learn to control differentiation. Their first target, too, is insulin-producing cells. While ITRI focuses downstream, Academia Sinica, Taiwan's premier collection of publicly funded science labs, is now ramping up a stem cell program focusing on understanding basic stem cell biology.

Challenges

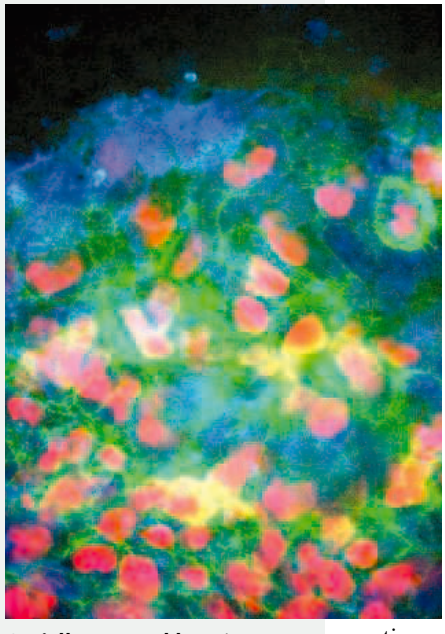
Although Asia's stem cell efforts are coming into their own, the region faces a number of challenges. Some worry that important stem cell research is going unpublished because of the intense interest in commercialization by Asian governments, companies, and researchers. Unlike Western biotech companies, which often seek the limelight, representatives of private companies in both Tai-

Asian Countries Permit Research, With Safeguards

Government officials, researchers, and ethicists in Asia readily link the region's general acceptance of research using human embryonic stem (ES) cells to its dominant Buddhist and Confucian religious-ethical traditions. But the countries of East Asia have also put a lot of thought, effort, and public debate into formulating policies that define researchers' responsibilities, as well as oversight mechanisms to ensure that guidelines are followed.

Although broadly similar, the policies adopted throughout the region differ in details. China, South Korea, Taiwan, and Singapore have all banned reproductive cloning with the intent of creating a child. All four regions also allow the derivation of ES cells from surplus in vitro fertilization (IVF) embryos obtained with informed consent; China, in addition, allows researchers to use embryos from aborted fetuses or miscarriages. South Korea's law stipulates that only embryos preserved for at least 5 years can be used. In each country except China, bioethics advisory committees have proposed national review boards to approve and oversee the derivation of new stem cell lines and each specific research project using them.

Singapore and China allow the creation of embryos through IVF for research purposes; South Korea and Taiwan forbid this. Countries are split on therapeutic cloning, or the use of adult somatic cells to create stem cells genetically matched to the donor. Singapore and China will allow it with the same oversight as for ES cells. South Korea has decided to restrict therapeutic cloning to a limited number of groups and solely for work that can't be done using typical ES cells. The country's national review board will decide which groups and projects qualify. Taiwan's advisory committee "split 50-50" on therapeutic cloning, says committee member Daniel Tsai, a physician on the faculty of National Taiwan University. It put off a decision pending further study.



Socially acceptable. Asian countries are less encumbered by the ethical dilemmas that have hamstrung research in the West.

Singapore, South Korea, and Taiwan incorporated societal views through high-level bioethics committees that held public hearings and made recommendations for the governments to codify into law. South Korea adopted a law governing ES cell derivation and research in December 2003. In September 2004, Singapore banned reproductive cloning but left pending the creation of a national review board. Under both laws, violators face prison sentences of up to 10 years or hefty fines or both. South Korea's review board is now being formed. Singapore's and Taiwan's need enabling legislation. For now, researchers using ES cells in Singapore must report their activities to the Ministry of Health. Until Taiwan passes legislation, says Tsai, institutions are trying to follow the recommendations of the bioethics committee; anyone violating the administrative ban on human cloning could lose a license to practice medicine or be forced out of an academic post.

Serious debate in China on stem cell research ethics began only in late 2001, after a team led by Chen Xigu of Zhongshan Medical University in Guangzhou claimed it had cloned embryos by inserting a child's DNA into an enucleated rabbit egg. Although the news was met with skepticism, and the team never published its results, the report set off a public storm. Galvanized by the furor, Chinese bioethicists held several meetings in 2002 and 2003, submitting the results to a newly formed interagency committee of the ministries of Health and Science and Technology. Issued in December 2003, the committee's "ethical guiding principles" are much less formal than other nations' regulations—they are fewer than 500 words long and specify no penalties for violation. Although the regulations are intended to "give researchers a lot of freedom," the bottom line is clear, says Deng Hongkui of Peking University: "There will be no reproductive cloning in China."

–D.N. AND C.C.M.

wan and South Korea were reluctant even to name their research topics to *Science*. Speaking under condition of anonymity, two Chinese researchers confessed they had not fully informed their granting agencies of what they were doing.

Deng Hongkui, a former New York University researcher known for his work on HIV, moved in 2001 to Peking University. Deng readily concedes that he has delayed submitting his research on the mechanisms of differentiation for publication for 2 years partly because his lab was preoccupied with the SARS emergency and partly, he says, because he wanted to secure worldwide intellectual property rights.

In China, researchers admit, the penchant for secrecy is heightened by rivalry and suspicion, which sometimes prevents groups from sharing data, expertise, and equipment as freely as their colleagues in the West. But they contend that this lack of communication is exacerbated by Asian researchers' continuing isolation from the scientific mainstream. Yang attributes some of this isolation to what he calls Western researchers' "inability to believe that top-rank research can come from developing nations in Asia." The biggest challenge facing the region "is not the lack of financial resources or good bench-level researchers but the lack of leaders," says Haifan Lin, a stem cell researcher at Duke University in Durham, North Carolina, who serves on a grant review committee at China's National Natural Science Foundation. All of these countries are trying to recruit researchers from outside their borders. Singapore has been the most aggressive, partly because it is so understaffed. Singapore also has advantages in recruiting nonnatives, as English is the language of commerce and government and the city is relatively cosmopolitan. "For me, it was Singapore or nothing," says ES Cell's Colman. Fifteen nations are represented on ES Cell's 25-person scientific team.

Some countries are already following China's lead and targeting expatriate sons and daughters. At Taiwan's Academia Sinica, most of the half-dozen Ph.D.-level researchers in the new stem cell group are Taiwanese or Chinese researchers returning from stints in the United States, the United Kingdom, or Australia. Group leader John Yu is a case in point. The former director of experimental hematology at Scripps Research Institute in La Jolla, California, Yu says he was lured back by the opportunity to get in on the ground floor of an exciting new effort and the chance to work in his native region.

Yu and other Asian scientists say they view these questions about leadership, openness, efficiency, and labor power as hurdles, not barriers, and are determined to overcome them. And they say their Western colleagues should expect to see more headline-grabbing research results come out of Asia in the next few years.

–DENNIS NORMILE AND CHARLES C. MANN

CREDIT: A. LASLETT, A. FILIPCZYK, AND M. PERA/MONASH INSTITUTE OF MEDICAL RESEARCH AND AUSTRALIAN STEM CELL CENTRE

The Unexpected Brains Behind Blood Vessel Growth

Two of the hottest fields in developmental biology—neural guidance and angiogenesis—are beginning to merge as scientists find that similar proteins control both processes

Whether in San Francisco or Singapore, almost everyone knows what the colors on a traffic light mean. But how did red, green, and yellow get chosen? It turns out railroad signals were already using these colors to guide trains. And the railroad industry may have gotten the idea from the electrical industry, which apparently used red to show that a motor was stopped and green to signal that it was running. When something works, why not use it more than once?

Evolution follows that principle too, as researchers studying the growth of blood vessels and nervous systems are beginning to appreciate. Scientists probing the development of the veins, arteries, and capillaries that guide nutrients and oxygen to cells are finding more and more evidence that the genes and proteins that were first discovered to guide growing nerve cells also direct blood vessels.

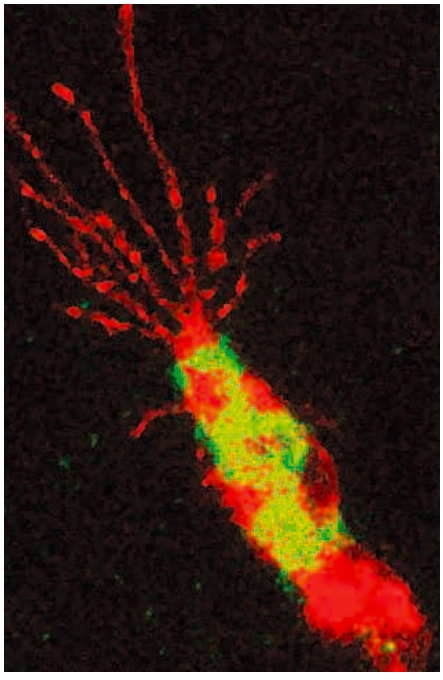
Decades of work by neuroscientists detailing the complex interactions of those cues is now giving researchers who study angiogenesis—the growth of blood vessels—a boost in their understanding of the vascular system. "Neurobiology has made an immeasurable contribution to angiogenesis," says David Anderson of the California Institute of Technology in Pasadena, who noticed some of the first overlaps. "All the insights we've gained from studying these signaling systems in the nervous system have put us in a much better position to understand how they work in the vascular system."

At the same time, one of the most powerful triggers of blood vessel growth, a protein called vascular endothelial growth factor (VEGF), is turning up in nerve cells and may play a key role in keeping them healthy and alive. "There is remarkable overlap in the use of these [signaling] systems," says David Ginty of Johns Hopkins University in Baltimore, Maryland.

These insights not only are inspiring a new respect for the complexity and precision of growing blood vessels, but they also have potential medical implications. Animal trials suggest that VEGF is a potential weapon against amyotrophic lateral sclerosis (ALS), an incurable disease that attacks nerves and gradually paralyzes its victims. And for those trying to control the growth of blood vessels—either to stop them from supporting cancerous tumors or to help them regrow after illness or injury—the nerve proteins offer a wealth of new targets to manipulate.

The tipping cell

One of the first signs of flirtation between the two fields came in 1998: Michael Klagsbrun of Children's Hospital in Boston and his colleagues reported in *Cell* that neuropilin, a cell surface protein originally identified as a receptor for a signal that guides growing nerves, also responds to VEGF (*Science*, 27 March 1998, p. 2042). Klagsbrun's observation "was an amazing discovery," Ginty says, although in hindsight it makes perfect sense, because both the blood vessel and nervous systems are "vast networks of complicated connections." Later that year, Anderson and his colleagues reported that another set of neuronal guidance molecules, cell surface proteins called Ephrin B2 and EphB4, were also present in the developing vascular system.



Looking for direction. The end of a developing blood vessel sends out sensory tentacles that resemble the growth cones of axons.

CREDIT: A. ABRAMSSON/KAROLINSKA INSTITUTE

Before these new observations, blood vessels were largely thought to form along a path of least resistance, without much active guidance. But the recent work paints a subtler picture, in which guidance molecules provide precise attractive and repulsive cues to specific growing vessels, notes Christer Betsholtz of the Karolinska Institute in Stockholm, Sweden. Work by Betsholtz and his colleagues revealed some of the first evidence for that precision. They showed in 2003 that specialized cells at the tip of developing blood vessels are attracted by slight changes in the concentration of VEGF. That reminded many biologists of what they see at the front of extending axons: the long extensions of a nerve cell that reach out and connect with other cells. "There are certainly some differences," says Ruediger Klein of the Max Planck Institute of Neurobiology in Martinsried, Germany, "but if you look at the pictures [from Betsholtz], the tip cells look very much like an axon's growth cone, extending and sensing the environment and responding to cues."

And the tip cells seem to respond to at least some of the same cues as growth cones. Last November in *Nature*, a group led by Anne Eichmann of the College of France in Paris described how those tips respond to netrins, a family of secreted proteins that help attract some axons and repel others during the formation of the spinal cord. How nerve cells react to netrins depends on which receptors they express, and, the new work shows, blood vessels can also react in different ways to the chemicals. Eichmann, with Peter Carmeliet of the University of Leuven in Belgium and Mark Tessier-Lavigne of Stanford University in California and their colleagues, reported that the gene for one of the previously identified netrin receptors, called *Unc5b*, is expressed in the tip cells of developing blood vessels. When the team created mice and zebrafish that made a faulty version of UNC5B, the vascular system of the mutant animals had far more sprouts and branches than normal—suggesting that the tip cells were impervious to a "stay away" signal from netrins. Indeed, the team subsequently showed that Netrin 1 causes the sprouts of rat blood vessels growing in culture to retract.

But that is not the whole story. In a paper published nearly simultaneously in the *Proceedings of the National Academy of Sciences*, Dean Li and his colleagues at the University of Utah, Salt Lake City, showed that Netrin 1 can also encourage the growth of new blood vessels, suggesting that the molecule may reprise its sometimes attractive, sometimes repellent role in the vascular system.

Eichmann and her colleagues have come across hints of other roles for netrins and their receptors in blood vessel development. They found that the UNC5B receptor is

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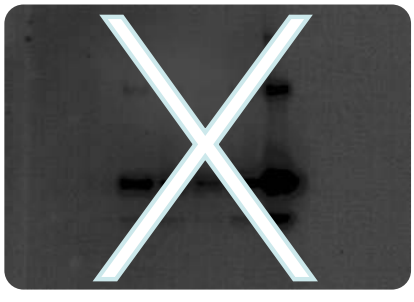

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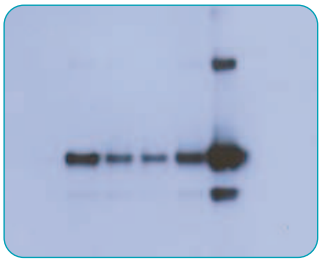

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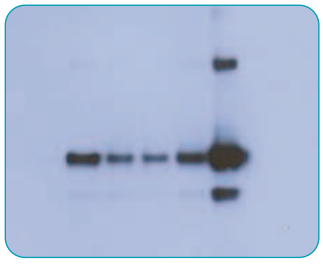

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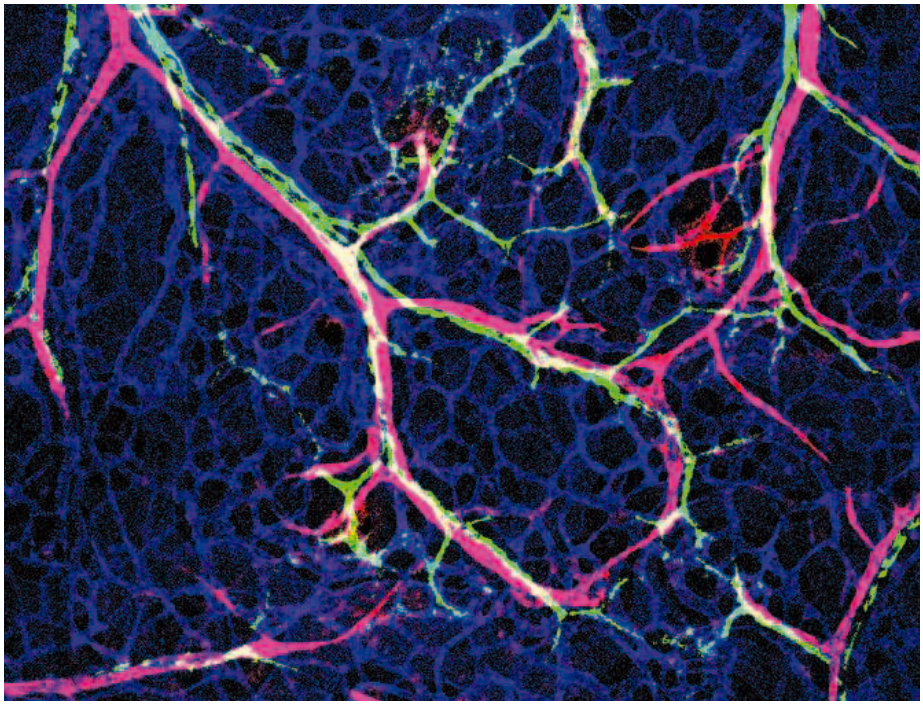
widely expressed in arteries, which deliver oxygen-rich blood to tissues, but it is apparently absent in veins, which return oxygen-depleted blood to the heart.

The early work on ephrin and Eph molecules from Anderson and his colleagues had showed a similar pattern. In their 1998 paper that established some of the first links between neuronal guidance and angiogenesis, the team showed that Ephrin B2 is expressed in arteries but not veins. Conversely, the ephrin receptor called EphB4 is expressed in veins but not arteries. These data were the first sign that arteries and veins are molecularly distinct at the earliest stages of development. Klein and his group confirmed that finding several months later and showed that the proteins could prompt the growth of new capillaries.



Follow me. In developing chick skin, arteries (red) align closely with nerves (green).

CREDIT: Y. MUKOYAMA AND D. ANDERSON/CALIFORNIA INSTITUTE OF TECHNOLOGY

Anderson and his colleagues found another bond between developing nerves and blood vessels. They showed that in the skin of developing chicks, arteries are guided in part by the development of nerves, whereas veins are not. They also studied mice lacking Semaphorin3A, one of the proteins that neuropilins recognize. These animals develop badly misdirected nerves, and their developing arteries followed the deviant paths of the nerves, providing more evidence that the systems are closely intertwined.

That observation is consistent with work on semaphorins by two other groups, one led by Ginty and the other by Brant Weinstein of the National Institute of Child Health and Human Development in Bethesda, Maryland. Each showed last year that semaphorins keep developing blood vessels on the straight and narrow. According to their research, zebrafish and mice lacking semaphorins or their receptors develop strikingly disorganized vessels.

A role in blood vessel growth for a fourth category of neuronal guidance molecules—the Slit proteins and their Robo receptors—may be emerging as well. In 2003, Jian-Guo Geng of the Shanghai Institutes for Biological Sciences at the Chinese Academy of Sciences in Shanghai and his colleagues reported that a wide variety of tumor cells produce a protein called Slit2, and that endothelial cells, the precursors of blood vessels, express the receptor Robo1. They suspect that the tumor cells might be using Slit proteins to attract new blood vessels to the growing tumor tissue. And in October, Roy Bicknell of Oxford University and his colleagues reported evidence in the *FASEB Journal* that a newly identified Robo receptor, which they call Robo4, is present in areas where new blood vessels are forming.

Receptive nerves

Neuroscientists are also learning from angiogenesis researchers. VEGF, the classic trigger of blood vessel growth, is showing up more and more in studies of nerve growth and development. The first clues to VEGF's neuronal role came from experiments in Carmeliet's lab at the University of Leuven in Belgium. To sort out some of the multiple roles VEGF plays in vascular development, Carmeliet and his colleagues created several strains of mutant mice that carried slightly altered versions of the protein. The mice seemed to develop normally but became ill as adults. "To our surprise, we found that they had motor neuron degeneration similar to that seen in ALS," says Carmeliet.

Normally, VEGF is expressed in response to low oxygen levels—it attracts new blood vessels to tissues that are short of oxygen. Carmeliet's mice carry a mutation that prevents that oxygen-dependent increase in expression, suggesting that perhaps a lack of VEGF leaves nerves vulnerable to hypoxia. Indeed, in studies of nerves in culture, introducing VEGF seemed to help the cells survive stressful conditions such as low oxygen or serum deprivations.

There are early hints that VEGF might play a role in some human ALS cases as well. In a study of 2000 people in England and Sweden, Carmeliet and his colleagues found that those carrying a certain version of the VEGF gene, one which seems to lower its overall production, were 1.8 times more likely to develop ALS than the general population.

Carmeliet and his colleagues have tested in animal models of ALS whether increasing production of VEGF combats the condition. In one rodent trial, they injected into muscles a rabies virus, which homes in on and infects nerve cells, modified to churn out VEGF. The mice that received the virus took longer to develop ALS-like symptoms and survived longer than their untreated counterparts. Working with a rat model of ALS, the team has also injected the VEGF protein directly into the cerebral fluid and documented similar benefits. The team is now preparing human trials, Carmeliet says, which could be under way within 2 years.

Angiogenesis researchers are hoping that the molecules that originally held the promise of regrowing severed or damaged nerves may pay off in another clinical area as well: the fight against cancer. These researchers have been attempting to fight tumors by cutting off their blood supply—essentially starving them to death. The finding that neural guidance molecules influence normal blood vessel growth has suggested a wealth of potential new targets, says Tessier-Lavigne: "There is every reason to believe [these molecules] will regulate pathological angiogenesis as well."

The discoveries in both fields may have even wider impact. Eichmann and her colleagues have shown that mice lacking neuropilin-2 have defects in their lymph systems. Similarly, in the 1 February issue of *Genes and Development*, Klein and his colleagues describe how mice lacking Ephrin B2 develop major defects in their lymphatic systems as well. Nature, it seems, has made the most of a good idea.

–GRETCHEN VOGEL

'Darwinian' Funding and the Demise of Physics and Chemistry

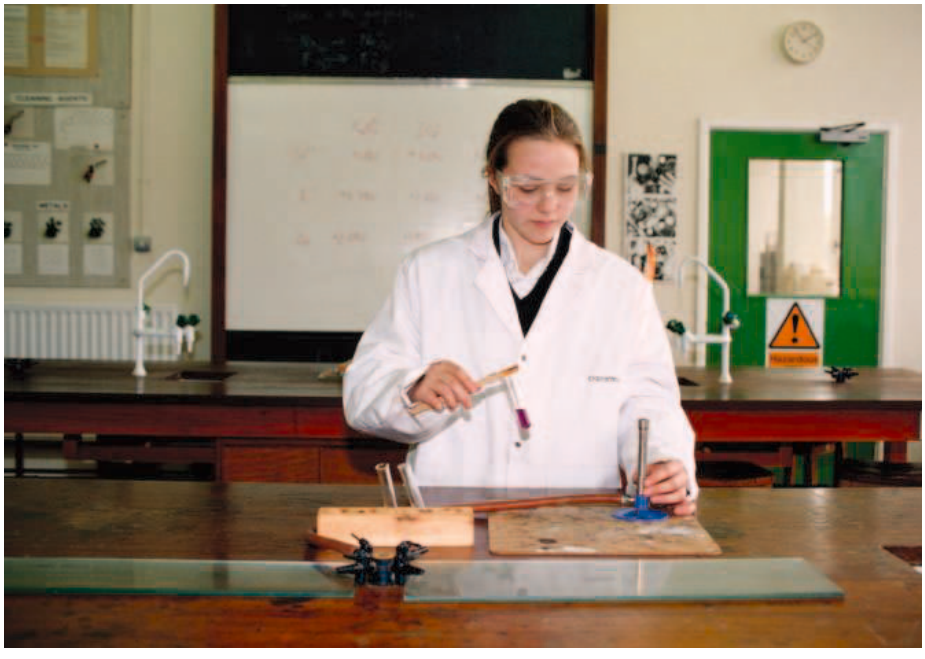
Britain's scheme to favor the highest-scoring research teams—abetted by other changes in society—is decimating chemistry and physics departments

CAMBRIDGE, U.K.—The word "university"—from the Latin *universitas*—suggests the whole, the world, or the universe. But is an institution still worthy of that moniker if it doesn't teach chemistry or physics? Universities in the United Kingdom seem to think so. Over the past decade, they have announced a steady stream of department closures, and now less than half of all U.K. universities offer undergraduate chemistry degrees. Physics has suffered a similar decline. "It's a disaster," says chemistry Nobelist Harry Kroto of the University of Sussex.

Department closures became headline news late last year when Exeter University announced plans to close its chemistry department, and Kroto threatened to hand back an honorary degree from the university. It was a surprising case particularly because Exeter's chemistry department was not failing: Almost all its work met a national standard of excellence, as judged by the 2001 Research Assessment Exercise (RAE), a government scheme that grades university departments. And during the 2004–05 academic year, Exeter had seen a 21% rise in applications to study chemistry. Nevertheless, the university's senate voted in December to close the chemistry department and concentrate on a new school of biosciences and on strengths in physics and sports science.

Ask researchers why this is happening, and they generally respond that the government, which is the main source of money for U.K. universities, is not providing enough for expensive lab-based courses such as physics and chemistry. This public contribution "has never been able to finance science departments to operate at even a minimum level," says Philip Kocienski, head of Leeds University's School of Chemistry. But other forces are at work, too. Demand for physics and chemistry classes has been steadily falling as students are lured into more career-specific courses such as sports science, forensic science, and media studies. And the once cozy world of British academia is now a competitive marketplace in which universities must vie with each other for government research money and attract as many students as possible to maintain their income. Some researchers suspect that current funding policies are designed to weed out the weak and concentrate resources in a smaller number of superdepartments. "It's a Darwinian exercise," says Kocienski.

The government has taken a hands-off approach so far, respecting the universities' autonomy. But the row over Exeter's withdrawal from chemistry has forced the government to rethink its neutrality. In December, then–Education Secretary Charles Clarke asked the Higher Education Funding Council for England (HEFCE) to look into ways to protect five strategic areas of study, one of which includes all of science, engineering, technology, and mathematics. Whether this will halt the closure of physical science departments nobody knows. One thing is certain: No new money will be available.



All alone. Fewer and fewer U.K. high school students want chemistry degrees.

CREDIT: GETTY IMAGES

Get 'em while they're young

No amount of new money would get around one critical fact: Physical sciences are not as popular among prospective university students as they once were. Although absolute numbers of applications have stayed fairly stable, Prime Minister Tony Blair's Labour government has successfully worked to increase the number of students going into higher education. As the total expanded, the fraction going into physical sciences grew smaller and smaller. (In the United Kingdom, students apply to universities to study a particular subject, and they specialize in their chosen major from the beginning.) "There is a serious supply-side problem," says metallurgist Graeme Davies, vice chancellor of the University of London.

What motivates teenagers to choose one course over another is not a simple question, but many blame science's declining appeal on the lack of good role models in the classroom. Britain's school system has long had a problem attracting science graduates into teaching; other careers offer much better salaries and opportunities for advancement. As a result, few high school pupils are taught physics or chemistry by teachers with degrees in those subjects. John Enderby, president of the Institute of Physics (IOP) in London, says the crisis in science departments is "a symptom of the underlying cause: We don't value teachers."

Other social incentives are at work, too. Few high school students see the benefit of studying a basic science. Meanwhile, television has made jobs in forensics, for example, seem glamorous, and universities now offer courses that appear to provide a fast track to that career. Member of Parliament Ian Gibson, former head of biological sciences at the University of East Anglia in Norwich, says university administrators "will teach anything to get students." Gibson, now Labour's chair of the House of Commons Science and Technology Committee, says

police chiefs have told his committee that they don't want such graduates. What they need are "good chemists and physicists." Simon Campbell, president of the Royal Society of Chemistry, says "it is up to us" to make careers in science attractive.

Follow the money

Attracting students is not enough to keep a department afloat, however, as Exeter's experience has shown. Many believe that government funding policies are quietly changing the shape of higher education by channeling research funding into science powerhouses while leaving other departments to founder.

Government funding to universities is distributed by HEFCE and partner councils in Scotland, Wales, and Northern Ireland. For the current academic year, HEFCE, by far the largest of the four councils, will distribute $11 billion to English higher education institutions, of which $7.2 billion goes in support of teaching and $2 billion for indirect costs associated with research. The teaching portion is divvied up according to how many students the university enrolls and how expensive their courses are to teach. So each humanities student earns a university $6600, while each undergraduate in lab-intensive subjects such as physics and chemistry, for example, wins the university 1.7 times as much ($11,000). Medics, dentists, and vets earn a fourfold boost ($26,000).

Many researchers argue that this extra funding is not enough to cover the costs of lab buildings, materials, and support staff. "Chemistry is expensive to teach," says Campbell, and HEFCE provides "woefully inadequate funding." Enderby agrees: "In all subjects the full cost of teaching is not met, but the shortfall is greatest for the laboratory sciences." HEFCE spokesperson Philip Walker counters that the allowances are based on a study of what universities actually spend. "We have to have a fair and transparent means to allocate the money," he says. In any event, Walker points out, once HEFCE has done its calculations, the money is given to the university as a lump sum. "Universities can allocate the money internally as they want." The implication is that Exeter itself bears the chief responsibility for the choices it made. "Exeter's chemistry department was not a dying animal," says Stephen Chapman, head of the School of Chemistry at Edinburgh University. "It was shot rather than left to die."

Academic cattle market

Departments that find they cannot manage with the teaching grant from HEFCE often end up subsidizing teaching from their research income. But not all departments have this luxury, as HEFCE research grants vary greatly in size depending on the quality of a department's research output. In 1992 HEFCE launched the RAE, its quality-control survey, which it repeats roughly every 6 years. Specialists in each subject rate the research in all university research departments and grade them on a scale from 1 (the lowest) to 5*, the score reserved for departments with "international excellence" in more than half of the work submitted for review. These grades have a major impact on funding, so before each new RAE, departments scramble to hire the hottest new researchers in the hope of bumping up their rating.

Most U.K. research departments cluster around the top end of the scale, with the peak of the curve around the boundary between grades 4 and 5. But following the 2001 RAE, many departments were shocked when the government decided to focus on the top achievers, pushing more of HEFCE's research funding into the highest-rated departments. Since that assessment, departments rated lower than 4 have received no research funding from HEFCE; those rated 5 and 5* get approximately three times as much per researcher as those rated 4. And since 2001, many 4-rated departments, such as chemistry at Exeter, have found themselves fighting for survival.

Although the RAE is a painful process, it's widely credited with having improved the quality of research in the United Kingdom. But many think it may have gone too far, and HEFCE is reviewing the system before the next RAE in 2008. "The RAE aims to starve out the weak, and it's been quite effective. But now it's cutting into flesh rather than fat," says Kocienski. "Vice chancellors are all too ready to use the RAE to cull expensive departments," adds Kroto.

Cooperation not competition?

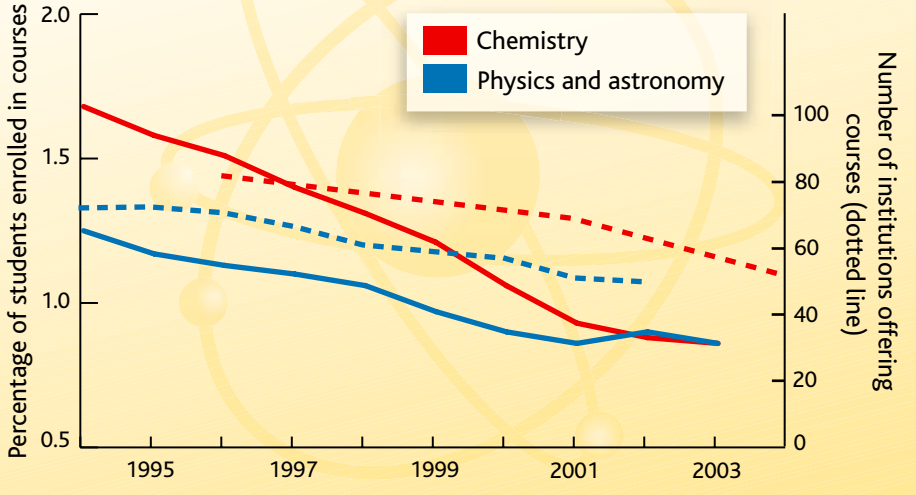
Gibson thinks the current crisis is the result of politicians forcing university administrators to think like business people and make decisions on purely financial grounds. "There is a lack of understanding among academic bigwigs about the needs of chemistry and physics," he says. Kocienski, voicing a pessimistic view, says the current total of about 40 chemistry departments may dwindle further to just 20: "I suspect that the government has this number in mind, too." The physics community is concerned that as closures continue, ever-larger swaths of the country will be left without any physics department. Students may have to travel farther from home to study physics, the IOP warns, and businesses will not be able to work with local researchers on R&D projects.

In Scotland, universities are already trying to counter the trend by taking a pragmatic approach: They are teaming science departments together for greater strength rather than letting the weakest go to the wall. Six Scottish physics departments have formed SUPA, the Scottish Universities Physics Alliance, and chemists from four universities will meld into two superdepartments: EastCHEM and WestCHEM. Each of these bodies will be entered into the RAE as a single department. Last November, these initiatives won $70 million for the next 4 years from Scottish funding bodies. "We have a vision of where we are going in chemistry and physics," says Edinburgh's Chapman. "We're not going to close things because one department is not doing well."

Other changes may be coming. Education Secretary Clarke's decision to consider protecting strategic subjects is a sign that the government may have concluded that it cannot govern higher education by a form of natural selection. "Do we need every department to be world beating?" asks Enderby: "No. Do we need a widespread education in physics? Yes."

–DANIEL CLERY

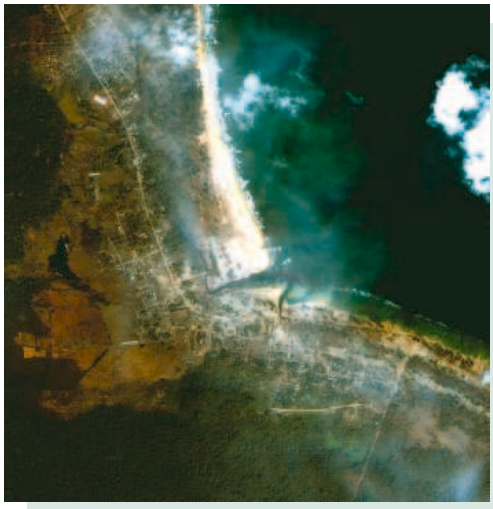
Physical Sciences' Declining Popularity



SOURCES: INSTITUTE OF PHYSICS; ROYAL SOCIETY OF CHEMISTRY

Random Samples

Edited by Jennifer Couzin



After the Earth Moved

New Delhi—Indian geologists are scrambling to remap the Andaman and Nicobar islands after the devastating earthquake and tsunami that struck South Asia on 26 December.

Prithvish Nag, India's surveyor general, said at a scientific meeting here last month that the 700-kilometer island chain in the Bay of Bengal has moved southeast toward Sumatra by as much as 1.25 meters and has been twisted in a counterclockwise direction. At the same time, the combination of sinking land and rising water levels has meant that the sea has swallowed roughly 1 vertical meter of coastline.

India plans to spend at least $25 million to document the island's new geomorphology, Nag said. The dozen or so Global Positioning System control locations on the islands needed to be recalibrated after having been thrown for a loop by the magnitude 9 earthquake. Nag said the remapping must be done quickly so that the government can provide advice on where to relocate residents now temporarily housed in refugee camps on the islands, which support a large air force base and a substantial navy presence.

The Burden of Sex

Sex has its repercussions, especially in the United States. There, premature death and disability linked to sexual behavior is triple that in other wealthy countries, researchers have found.

Previously, epidemiologists have counted cases of sexually transmitted diseases (STDs) and deaths caused by them. But that's only part of the picture, says Shahul Ebrahim of the Centers for Disease Control and Prevention in Atlanta, Georgia. Some STDs boost the risk of a second infection or cervical cancer or cause infertility. Ebrahim and colleagues also included premature deliveries and unintended pregnancies, which cause psychological distress, as causes of disability.

They found that U.S. women bear more of the brunt than men. In particular, they suffer from curable infections, such as chlamydia, and their consequences, particularly infertility, the team reports in the February issue of *Sexually Transmitted Infections*. For men, HIV is by far the leading problem. The heavy toll is not surprising, given the higher incidence of HIV and unintended pregnancies in the U.S., says epidemiologist Ward Cates, president of Family Health International, a nonprofit in Research Triange Park, North Carolina.

A Nose for Survival

Encountering a robber crab might send you running in the opposite direction. The world's largest land-living arthropod weighs up to 4 kilograms and steals anything it can nab with its formidable pincers. It turns out the crab has another unusual feature: It has evolved to smell on land much the way insects do. The finding is "cool," says Leslie Vosshall, an olfactory researcher at Rockefeller University in New York City.

Bill Hansson, a chemical ecologist at the Swedish University of Agricultural Sciences in Alnarp, Sweden, measured the electrical activity of olfactory neurons in the crab's short antennae while puffing scents over these sensory organs. The electrical activity spurred by certain odors was the same in robber crabs as in insects. And like insects, the crabs weren't drawn to just any smell; they favored chemicals, like dimethyltrisulfide, that are released from decaying meat.

Hansson's team also found that the short antennae are structurally similar to those of insects and have a wrinkly lining, which probably helps sense odors. This is a "great example of convergent evolution," says Hansson. The researchers report their results in the 26 January issue of *Current Biology*.

Da Vinci Discovery

Five small rooms wedged between a Florentine military institute and a monastery were apparently once a canvas for Leonardo Da Vinci. Last month, three researchers at the Italian Institute of Military Geography announced that they'd stumbled upon mural paintings after an unused staircase was demolished. The soaring birds (see picture) closely resemble drawings by the 16th century master (inset). "To us, there seems to be no doubt," says Roberto Manescalchi, one of the discoverers. Superimposing images of the wall paintings on drawings in Leonardo's *Codex Atlanticus*, he says, revealed a perfect match.

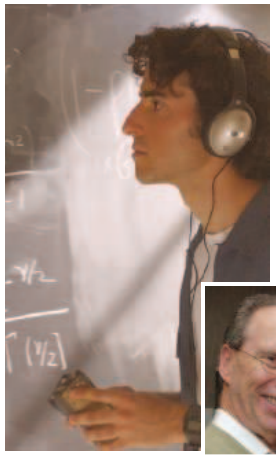
Still, questions remain: Several experts have since visited the rooms, but they can't resolve whether the murals are by Leonardo himself or one of his pupils, says Cristina Acidini, head of an institute for the restoration of works of art in Florence operated by Italy's Ministry of Culture.

CREDITS (TOP TO BOTTOM): SPACE IMAGING; MARCUS STENSMYR AND BILL S. HANSSON; *CODEXATLANTICUS*

Edited by Yudhijit Bhattacharjee

TWO CULTURES

Math is hip. After 37 years at the California Institute of Technology, mathematician Gary Lorden finally has students asking him for his autograph. But it's a television show, not his lectures, that has made him a celebrity.



The chair of Caltech's math department, Lorden (inset) is an adviser to *Numb3rs*, a cop drama on CBS featuring mathematician Charlie (David Krumholtz), who helps his detective brother solve crimes. "It's kind of like Sherlock Holmes on steroids, where the steroids are mathematics and computer science," says Lorden. "In one episode, they're tracing the outbreak of a disease from a kind of terrorist attack. Another one involves predicting bank robbers' behavior: where and when they are going to hit next."

Lorden is supposed to lend authenticity to the mathematics on the show, which he's done so far by suggesting changes to the dialogue and having graduate students write equations on Charlie's blackboards. Lorden hopes that *Numb3rs* will help students realize that mathematics isn't just for fusty academics.

Got any tips for this page? E-mail people@aaas.org

AWARDS

Academy honors. A veteran of the campaign to wipe out smallpox has been awarded the National Academy of Sciences' (NAS's) highest honor, the Public Welfare Medal.

Epidemiologist William H. Foege's work on smallpox eradication in Africa in the 1960s led to the successful "ring vaccination" strategy of inoculating close contacts of infected people. Foege later directed the Centers for Disease Control and Prevention, steered the international Task Force for Child Survival and Development, and worked on eradicating Guinea worm disease and river blindness at the Carter Center. In 1999 he joined the Bill and Melinda Gates Foundation, where he helped start programs including hepatitis B immunization and AIDS vaccine development. His work "has changed the world as we know it," says NAS home secretary John Brauman.

Now retired, Foege will be honored 2 May along with 17 other winners of various academy awards.

Crafoord prize. A transatlantic trio of cosmologists has won the 2005 Crafoord Prize, awarded by the Royal Swedish Academy of Sciences.

Princeton University's James Gunn and James Peebles and Cambridge University's Martin Rees will share the $500,000 prize for work on how the universe evolved from a smooth primordial soup of particles and radiation into the present cacophony of galaxies and clusters. All three are theorists, but Gunn is also lead project scientist of the Sloan Digital Sky Survey, the largest project to date to map the three-dimensional distribution of galaxies.

"These are all very large names in cosmology," says theoretical astrophysicist Vincent Icke of Leiden University in the Netherlands. Their career contributions, he explains, have been "a real boon to the field."

JOBS

Moving on. In a different political climate, chemistry Nobelist Peter Agre says he might have been headed for the National Institutes of Health. Instead, the outspoken Agre—a vigorous supporter of Democrat John Kerry in last fall's presidential campaign—is heading to Durham, North Carolina, to become Duke University Medical Center's vice chancellor for science and technology, a newly created position that combines advising with advocacy.

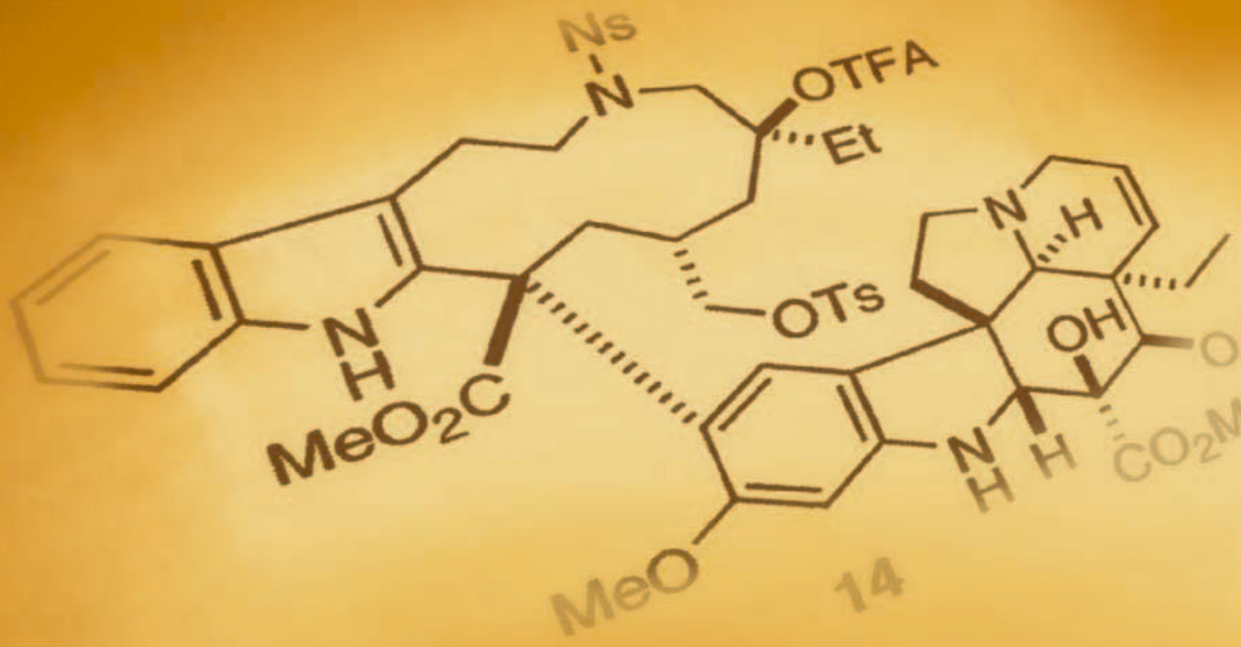
A professor at Johns Hopkins University in Baltimore, Maryland, since 1981, Agre says he was one of three candidates under consideration to be director of the National Heart, Lung, and Blood Institute (NHLBI) before dropping out last summer because he couldn't stomach the Bush Administration's stance on key science issues such as stem cell research. Last week Elizabeth Nabel accepted the position at NHLBI (*Science*, 28 January, p. 495).

But Agre insists he is "not going to Duke in any partisan way." Instead, he'll help set research priorities for the medical center, serve as the university's spokesperson on key science policy issues, and act as a "cheerleader for science" by promoting science literacy among the general public. "I have a weakness for championing causes," he says.

PIONEERS

A flood of interest. Since the tsunami devastated South Asia, Evan Variano, a graduate student in civil and environmental engineering at Cornell University, has taught more than 500 middle school and high school students how tsunamis develop and travel. Variano sets up a narrow, water-filled tube with a balloon inside, then inflates the balloon. The result is a sudden displacement of water, sending tsunami-like waves to the far end, where the amount of flooding depends on the shape of a miniature coastline. It works better than a lecture, he says: "The demo catches their attention, and they are curious to know more."

CREDITS (TOP TO BOTTOM): PARAMOUNT PICTURES CORP.; (INSET), BOB PAZ/CALTECH; DUKE UNIVERSITY MEDICAL CENTER; SOCIETY FOR ACADEMIC EMERGENCY MEDICINE; KEVIN STEARNS/CORNELL UNIVERSITY



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Evolution Versus Invention

THE PAPERS BY R. GELMAN AND C. R. GALLISTEL ("Language and the origin of numerical concepts," Viewpoint, p. 441), P. Pica *et al.* ("Exact and approximate arithmetic in an Amazonian indigene group," Report, p. 499), and P. Gordon ("Numerical cognition without words: evidence from Amazonia," Report, p. 496) in the Special Issue on Cognition and Behavior (15 Oct. 2004), taken jointly, establish two key points: Animals as well as humans have an analog system for representing numeracy. The analog system, although yielding only approximate answers, supports complex numerical computations including comparison, addition, and subtraction.

Hunter-gatherer groups, whose languages contain only a few number words ("one, two, many" in one case; "one" to "five" in another), pose a problem. Despite having number words, they perform only approximate numerical calculations, even when problems contain only numbers for which they have words.

Despite their interest and clarity, we suggest that the above-mentioned papers overlook two key points. First, hunter-gatherer number words are not comparable to our own. Even the smattering of data reported by these authors indicates that hunter-gatherer number words name approximate magnitudes, not exact sets of objects as ours do. There is then no paradox in the fact that hunter-gatherers perform only approximate numerical calculations.

Second, it is not language per se that is critical for understanding the transition from analog to digital numeracy, but the change from foraging that did not require exact numbers to technologies that did. Humans, as hunter-gatherers, evolved a system for analog numeracy [(*1*), p. 29]. When forced to adopt new technologies—pastoralism, gardening, trade or barter, and ultimately full-time farming—they then invented systems for representing exact numeracy: dots, bars, and a shell standing for zero (Mayan) (*2*); ropes and knots (Incas) (*3*); fingers and fists (sub-Saharan tribes) (*4*); the abacus (Chinese) (*5*); Roman and Arabic numerals; and so forth. The systems are all combinatorial: The use of a base (e.g., 5, 10, 20) permits forming new numbers by combining existing numbers. They are also recursive: New numbers are formed by adding one. Both recursion and a combinatorial approach are long-standing human capacities evident in, for example, both language and (to a lesser extent) social behavior.

Conceivably, the same contingencies led both to the invention of written language and to exact numeracy. Writing arose as a belated consequence of the transition from foraging to agriculture. Farming resulted in surplus goods, necessitating a system for marking the goods, identifying ownership of casks of oil and the like [(*1*), p. 8].

Although hunter-gatherers had little need for exact numbers, one can imagine that no owner of stored goods would wish to receive three casks of oil when he had stored four, nor would the individual who had stored the goods wish to return five casks when he had stored four.

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References
1. D. Premack, A. Premack, *Original Intelligence* (McGraw-Hill, New York, 2003).
2. M. D. Coe, *Breaking the Maya Code* (Thames & Hudson, London, 1999).
3. M. Ascher, R. Ascher, *Code of the Quipi: A Study in Media, Mathematics, and Culture* (Univ. of Michigan, Ann Arbor, MI, 1981).
4. P. Gerdes, *Historia Math.* **21**, 345 (1994).
5. Y. Li, S. Ran Du, *Chinese Mathematics, a Concise History*, translated by J. N. Grossly, A. W. C. Lun (Clarendon Press, Oxford, UK, 1987).

Elephants, Ecology, and Nonequilibrium?

ELEPHANTS AND THRIPS MAY HAVE SOMETHING in common: It has been proposed that elephants in Africa do not reach carrying capacity because they inhabit "nonequilibrium" ecosystems with highly variable rainfall ("Space—the final frontier for economists and elephants," E. Bulte *et al.*, Perspectives, 15 Oct., p. 420). Similarly, it has been proposed that thrips in Australia do not reach a carrying capacity because of climatic fluctuations (*1*). The nonequilibrium (density-independent) ideas of the 1950s are being reworked as "state-of-the-art" ecological theory by Bulte *et al.* We should remember, however, that a more sophisticated analysis (*2*) of the same thrip populations revealed strongly density-dependent population change and hence a carrying capacity.

The suggestion that multispecies systems are unlikely to show density dependence is erroneous. In contrast, evidence is emerging of the very widespread occurrence of density dependence (*3*), even in complex marine systems (*4*, *5*). Detection of such effects typically takes over four generations (*6*); well over a hundred years might be needed to detect density dependence in an elephant population.

CREDIT: ORLA SMITH/*SCIENCE*

The application of nonequilibrium hypotheses to savannah has been challenged on theoretical and empirical grounds. Models indicate that herbivores in semi-arid areas are in long-term equilibrium with a subset of their resources (*7*). Competitive regulation is now very clear in a number of species of large herbivores in Africa, including wildebeest (*8*). A review (*9*) concluded there was no evidence of a paradigm shift to a nonequilibrium perspective among those researching grasslands. Large species are unlikely to exhibit metapopulation dynamics (*10*).

The harvesting of elephants is, rightly, controversial. We suggest that the nonequilibrium perspective is unlikely to clarify how their populations might respond to management.

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References
1. H. G. Andrewartha, L. C. Birch, *The Distribution and Abundance of Animals* (Univ. of Chicago Press, Chicago, IL, 1954).
2. F. E. Smith, *Ecology* **42**, 403 (1961).
3. R. Lande *et al.*, *Am. Nat.* **159**, 321 (2002).
4. M. S. Webster, *Ecology* **85**, 986 (2004).
5. J. S. Shima, *Oecologia* **126**, 58 (2001).
6. P. Rothery, in *Insect Populations in Theory and Practice*, P. J. Dempster, I. F. G. McLean, Eds. (Kluwer Academic, Dordrecht, 1998), pp. 97–133.

7. A. W. Illius, T. G. O'Connor, *Oikos* **89**, 283 (2000).
8. A. R. E. Sinclair, C. J. Krebs, in *Wildlife Population Growth Rates*, R. M. Sibly, J. Hone, T. H. Clutton-Brock, Eds. (Cambridge Univ. Press, Cambridge, 2003), pp. 127–147.
9. D. D. Briske, S. D. Fuhlendorf, F. E. Smeins, *J. Appl. Ecol.* **40**, 610 (2003).
10. S. Harrison, in *Large-Scale Ecology and Conservation Biology*, P. J. Edwards, R. M. May , N. R. Webb, Eds. (Blackwell Scientific Publications, Oxford, UK, 1994), pp. 111–128.

IT IS A PLEASURE TO SEE ECONOMISTS BORROWING from ecology ("Space—the final frontier for economists and elephants," E. Bulte *et al*., Perspectives, 15 Oct., p. 420) because the traffic has usually been in the other direction. But Bulte *et al*. are surely throwing the elephant out with the bathwater when they assert that equilibrium ideas in population ecology are often false. We know that temporal environmental variation prevents plant-herbivore systems from reaching a stable equilibrium, but we also know what to do about it, in theory, if our aim is to harvest such populations sustainably (*1*). "Nonequilibrium," on the other hand, is a shibboleth often used to invoke some mysterious dynamical regime in which herbivores are somehow not coupled to the dynamics of their resources (*2*). The reality is simpler and more conventional than that. Spatial environmental variation results in a patchwork of resources that vary in quality and accessibility. A subset, the key resources (*3*), is what the herbivore population depends on to get through the dry season, when mortality threatens. Modeling shows that, despite short-term fluctuation due to temporal stochasticity, the herbivore population is in long-term equilibrium with these key resources. In other words, herbivore population size depends largely on the environment's endowment of key resources and hardly at all on the remaining parts of the habitat (*4*). Space may be the final frontier, but the challenge is to identify and manage the key resource, or equilibrial, parts of the system, rather than to worry about the irrelevant nonequilibrium remainder. By characterizing the entire system as nonequilibrium, and failing to make the distinction between types of resources, the nonequilibrium paradigm suffers from the problem of not seeing the trees for the wood.

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References
1. R. Lande *et al.*, *Ecology* **78**, 1341 (1997).
2. A. W. Illius, T. G. O'Connor, *Ecol. Applic.* **9**, 798 (1999).
3. I. Scoones, *J. Arid Envir.* **9**, 221 (1995).
4. A. W. Illius, T. G. O'Connor, *Oikos* **89**, 283 (2000).

Response

WE AGREE WITH ILLIUS AND HAMBLER *ET AL.* that density-dependent effects are important in the population dynamics of many species but differ with their interpretation of the term "nonequilibrium." A more important point, however, is that the simplistic bioeconomic models that we critiqued in our Perspective do not acknowledge the many other, complex interactions, apart from density dependence, that can also influence population size, nor the fact that density-dependent effects are only dominant at certain spatial scales (*1*).

We used the term "nonequilibrium" in its broadest sense to mean "not-at-equilibrium," rather than to imply that equilibrating, density-dependent processes are not important. It is well recognized in the ecological literature that environmental stochasticity can affect population size (*1*), but it is only a very small subset of nonequilibrium theories—those termed "disequilibrium"—that assert that environmental variability can completely override the effects of biotic interactions. Rangeland ecologists, for example, have argued that herbivore population density is largely determined by extreme events like drought, which in semi-arid savannas are frequent enough to keep populations at levels that are too low for density-dependent effects like competition to operate (*2*).

This disequilibrium viewpoint is extreme, however, and most authors now agree that both density-dependent and environmental variables affect population size (*3*). Although density-dependent effects might cause populations to tend toward an equilibrium, it is likely that the position of the equilibrium will change over time and that factors such as rainfall, fire, disease, or human influence deflect populations away from a possible equilibrium or "carrying capacity." Rather, population size may be envisaged as varying around an equilibrium point (perhaps more usefully termed an "attractor") and can move between two or more domains of attraction.

The debate in nonequilibrium ecology has thus moved beyond the question of whether density-dependent or environmental variables are most important. Since the early 1990s, discussion has focused on identifying the scales at which different processes predominate, and how to combine this knowledge in ways that are ecologically meaningful (*4*). Recent advances in hierarchical modeling, for example, integrate nested processes in a spatially explicit, hierarchical framework (*5*). Advances like these may provide the basis for spatially defined—and more ecologically realistic—bioeconomic models in the future.

In the meantime, equilibrium models with density-dependent factors can provide an approximation of reality at certain (species specific) spatial scales. The challenge is for economists and ecologists to work together to develop models that not only are theoretically rigorous but also incorporate appropriate spatial and temporal complexities of changing environments.

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References
1. D. L. DeAngelis, J. C. Waterhouse, *Ecol. Monogr.* **57**, 1 (1987).
2. R. H. Behnke Jr., I. Scoones, C. Kerven, *Range Ecology at Disequilibrium: New Models of Natural Variability and Pastoral Adaptation in African Savannas* (Overseas Development Institute, London, 1993).
3. J. Wu, O. L. Loucks, *Q. Rev. Biol.* **70**, 439 (1995).
4. S. Levin, *Ecology* **73**, 1943 (1992).
5. J. Wu, J. L. David, *Ecol. Model.* **153**, 7 (2002).

National Environmental Policy Act at 35

1 JANUARY 2005 WAS THE 35TH ANNIVERSARY of the signing into law of the U.S. National Environmental Policy Act (NEPA). It has since been copied and enacted in many local U.S. jurisdictions and around the world, so we believe this is the proper time to list some of its accomplishments and continuing problems.

First, the existence of the statute and its implementing regulations have required U.S. agencies to at least acknowledge that there are environmental consequences of their actions. Second, the existence and publication of NEPA Environmental Analyses (EA) or Environmental Impact Statements (EIS) have provided for much more public input into decision-making.

But NEPA was designed to do more. It was meant to force agencies to "insure the integrated use of the natural and social sciences… in planning and decision-making." The U.S. Council on Environmental Quality (CEQ) regulations for NEPA implementation say that "NEPA's purpose is not to generate paperwork—even excellent paperwork—but to foster excellent action," and to lead agencies toward "actions that protect, restore, and enhance the environment" (*1*). That goal is frequently lost in the admittedly difficult process of producing an EA or EIS.

In 1996, the average EIS was 570 pages, although the CEQ regulations state it should be only 150 pages, or 300 for complex projects (*1*, *2*). This mass of paper is frequently unnecessary, as it is no more than the description of the existing environment (*3*).

There are an enormous amount of environmental data already gathered on almost all of the United States. If these data were collected in one or more depositories, they could be reused and reduce the time and effort devoted to writing EISs (*4*) and could serve as a quality control mechanism for the statement produced.

NEPA explicitly states that agencies are to use an interdisciplinary approach to their work, but this is not apparent in current EISs. The problem is that, despite years of effort and the development of university programs that claim to teach interdisciplinary environmental research, the ability to perform it in the real world of deadlines and finite resources does not yet exist. Another problem is the separation of "social" impacts from "environmental" impacts in EISs. The underlying principle of NEPA is that all impacts of a project are eventually social, as they ultimately affect people. Evaluation of the cumulative effects of several projects is also missing from most EISs, which devote the majority of their analysis to the current project (*5*).

Over the past 35 years, NEPA has greatly improved the quality of U.S. governmental decisions regarding the environment and enhanced public participation in the process, but there are still aspects that need research and implementation if NEPA is to achieve its objectives.

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References
1. 50 C.F.R. § 1500.1.
2. B. C. Karkkainen, *Columbia Law Rev.* **102**, 903 (2002).
3. D. Bear, *Nat. Resour. J.* **43**, 4 (2003).
4. J. F. DiMento, H. Ingram, *Nat. Resour. J.*, in press.
5. N. N. Mccold, J.W. Saulsbury, *Environ. Manage.* **20** (no. 5), 767 (1996).

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Long-Lived *Drosophila* with Overexpressed dFOXO in Adult Fat Body"

Marc Tatar

Giannakou *et al.* (Brevia, 16 July 2004, p. 361) reported that dFOXO overexpression extends *Drosophila* life-span. However, mortality was similar among experimental and control groups in replicate trials, except for one week when there were many deaths in control adults. This early event reduces the control survivorship and is mistaken for evidence that aging is retarded by overexpression of dFOXO.
Full text at www.sciencemag.org/cgi/content/full/307/5710/675a

RESPONSE TO COMMENT ON "Long-Lived *Drosophila* with Overexpressed dFOXO in Adult Fat Body"

Maria E. Giannakou, Martin Gross, Martin A. Junger, Ernst Hafen, Sally J. Leevers, Linda Partridge

Tatar suggests that the conclusions of our study are erroneous due to excessive mortality in young control flies. We show that, both in our published results and in other results we had at the time of publication, there is a highly reproducible increase in overall survival and maximum life-span of females with dFOXO overexpression in fat body.
Full text at www.sciencemag.org/cgi/content/full/307/5710/675b

EVOLUTION

Voyage to the Bottom of the Tree

Charles Francis Delwiche

Men dreme alday of owles and of apes,
And of many a maze therwithal.
–Geoffrey Chaucer, *The Canterbury Tales*

Biology is a science with many stories. Each organism is a unique product of its evolutionary history, its adaptation to its environmental niche, and the constraints imposed by the laws of physics. With roughly 1.7 million known species, each one of which is enormously complex in its own right, there is a bewildering array of curiosities, exceptions, and oddballs. Given this riot of biological diversity, it can hardly be surprising that there has been a trend toward the "model systems" approach, which picks a handful of species to study in great detail. After all, it would be a great accomplishment to fully understand just one species. But model systems can only take us so far; to understand life it is necessary to grapple with its full diversity, including that which is unfamiliar or unseen. In a very real sense, each species has its own tale to tell.

In *The Ancestor's Tale,* Richard Dawkins approaches the topic of biological diversity by descending the tree of life one branch at a time, starting from the extant twig of six billion individual humans. At some point in the not-too-distant past (perhaps 6 million years ago), our ancestors along with those of chimpanzees and bonobos were members of a single species, neither human nor chimpanzee. Dawkins calls this species a concestor (for "common ancestor"). He traces our lineage back through progressively more inclusive and stout branches to the three main trunks and finally to the root of the tree of life. Describing (and numbering) concestors, Dawkins identifies 39 waypoints on this backward journey through time. At each of these "rendezvous," he stops to discuss the ancient common ancestor. Figures depict the topology of the branches joining our own lineage, provide the probable dates, and include tiny images of representative members of the descendant clades. In the Weidenfeld and Nicolson edition (which I read), these images are generally beautiful photographs; in the Houghton Mifflin version, they have been replaced with very simple line drawings. Readers of the U.S. edition will also find, unfortunately, that it lacks the often striking, full-page, color reconstructions Malcolm Godwin prepared of most concestors—and that all of the illustrations it does contain are presented in black and white.

Leading to lungfish and us. Dawkins identifies the common ancestor of lungfish and terrestrial vertebrates as a lobe-finned fish, the concestor reconstructed here.

The subject is timely. Although even a decade ago the structure of the tree of life was largely a matter of speculation, huge advances have been made in recent years, and the tree is now beginning to come into sharp focus. Only a handful of the branch points covered in the book remain in serious question, and these are clearly identified. Dawkins's task was simplified by the fact that humans are members of a relatively depauperate lineage that has been subject to intensive study. Other parts of the tree remain much more poorly understood, but for the first time at least the fiber of our own ancestry can be traced with some certainty all the way back to the earliest recognizable forms of life. Even for one accustomed to thinking about phylogeny and deep time, it is remarkable to ponder that unbroken line of descent, stretching over billions of years and extending back to, well, primordial ooze.

By its very nature, an accumulating phylogeny of life on Earth lacks the focused clarity of Dawkins's "selfish gene"—one of the strongest metaphors in popular science and the subject of one of the most important popular-science books ever (*1*). *The Ancestor's Tale* is loosely modeled after Chaucer's *The Canterbury Tales,* in which a group of pilgrims tell tales on their journey to Canterbury. Dawkins uses this as an extended metaphor, envisioning each branch of the tree of life as a group of pilgrims that joins us on our journey into the past. As each group joins the main assemblage, after describing the group and the reconstructed concestor, he relates stories that are inspired by that group, using them to illustrate fundamental biological principles. These tales are generally interesting and sometimes brilliant. With a couple of harmless exceptions, he wisely resists the urge to tell these stories from the animal's perspective, commenting, "[others] might get away with it, but not me." The metaphor of a growing pilgrimage really does help envision relationships, and it throws into stark relief the difference between "relict" groups, like monotremes (egg-laying mammals), that consist of a few survivors of an ancient lineage and major groups, like sauropsids (birds and living "reptiles"), that have undergone vast diversification.

The Chaucerian theme is cute and a lot of fun, but it leads to titles of the individual stories such as "Prologue to the Galapagos Finch's Tale" (an outstanding essay on the potential rapidity of adaptative evolution) and "The Brine Shrimp's Tale" (a highly speculative essay on the origin of a dorsal versus ventral nerve cord). The tales' titles, unfortunately, often convey next to nothing about their content, a circumstance that ultimately proves rather confusing.

Although in its entirety Dawkins's narrative follows an elegant arc, each individual tale follows its own distinct path. Herein lies the book's greatest problem, as many of the tales wander a fair bit along the way. What is more, Dawkins distracts the reader with many marginal notes (often more than one per page), some of which are quite extensive. The effect is the textual equivalent of trying to keep track of the epicycles in a geocentric

The Ancestor's Tale
A Pilgrimage to the Dawn of Life
by Richard Dawkins
Weidenfeld and Nicolson, London, 2004. 528 pp. £25. ISBN 0-297-82503-8.

The Ancestor's Tale
A Pilgrimage to the Dawn of Evolution
Houghton Mifflin, Boston, 2004. 687 pp. $28. ISBN 0-618-00583-8.

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model of the solar system. Add to that the cryptic titles of the tales and one becomes downright dizzy. Dawkins has written another wonderful book, but the manuscript would have benefited from a good, firm edit. Had the text been reduced by a third and the marginal notes either incorporated in the main text or eliminated, *The Ancestor's Tale* could have become a true classic. It should also be noted that Dawkins is an entertaining author precisely because he is not afraid to express opinions. However one might feel about the particulars, at times these opinions become downright caustic, and they trivialize the tales in which they appear. Conservative readers might risk an aneurysm.

The taxonomic scope of *The Ancestor's Tale* is strongly affected by the author's understandable decision to follow human ancestry. We are animals, so this is fundamentally a book about animals (i.e., metazoans). In the one chapter on plants, Dawkins comments, "I ended a previous tale by remarking what delight it is to be a zoologist at such a time. I could have said the same about being a botanist. What a pleasure it would be to demonstrate Deep Green [(*2*)] to Joseph Hooker—in the company of his close friend Charles Darwin. I almost weep to think about it." Nonetheless, in practice Dawkins seems to follow another great philosopher of science, Tom Weller, who said, "The evolution of plants is an important chapter in the history of life. However, it's a pretty dull chapter, so we'll skip it." (*3*) Furthermore, to my astonishment, the Archaea—a third of all life—are not allotted a single tale.

But so be it. Dawkins is an enormously talented author, and *The Ancestor's Tale* is expansive, current, and authoritative. There are, of course, technical errors and dubious assertions to be found. Few texts of such scope are without them. These flaws, however, are mostly minor, and the book avoids many pitfalls that have trapped other authors. It would be an excellent choice for an undergraduate honors seminar in zoology and could serve a graduate student well in preparation for oral exams. It is also entertaining, witty, and—at least in the "Pilgrimage to the Dawn of Life" version—beautifully illustrated. If it still leaves room for the botanists and microbiologists of the world to present their perspective on the tree of life, who am I to complain?

References and Notes

1. R. Dawkins, *The Selfish Gene* (Oxford Univ. Press, Oxford, 1976).
2. "Deep Green" refers to a project to reconstruct the phylogeny of green plants and to a hyperbolic visualization of that phylogenetic tree, available at http://ucjeps.berkeley.edu/map2.html.
3. T. Weller, *Science Made Stupid* (Houghton Mifflin, Boston, 1985).

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EVOLUTION

Seeing the Forest for the Trees

Scott J. Steppan

Assembling the Tree of Life presents a preliminary view of one of the grand enterprises of modern science, resolving the phylogeny of all life. Imagine a vast tree whose myriad branches lead to millions of leaves. Each leaf, itself composed of innumerable parts, represents an individual species in the history of life, and the tree stands billions of years tall. Revealing that tree is the shared vision of the world's systematists, but for now it remains a dream. We do not know what the whole "Tree of Life" looks like. We can only see parts of it, and our situation is worse than that of the proverbial three blind men trying to describe an elephant. Thousands of us work on particular branches, which are hidden from one another in a mist. This volume, the product of a 2002 symposium by the same name held at the American Museum of Natural History (AMNH) in New York, seeks to blow away the mist and reveal the structure of the whole Tree and, in doing so, galvanize the systematics community toward unifying its goals.

Assembling the Tree of Life
Joel Cracraft and Michael J. Donoghue, Eds.

Oxford University Press, New York, 2004. 592 pp. $59.95, £36.50. ISBN 0-19-517234-5.

A complete Tree of Life (hereafter "Tree") holds enormous promise for many fields of science, but the task of revealing it is an enormous undertaking—one that requires more data than the Human Genome Project (just one leaf on the Tree) and orders of magnitude more computation. Even small parts are difficult; as Michael Whiting notes, "A child can tell a beetle from a wasp from a butterfly, but even the entomologically erudite is left pondering which two insects are most closely related." The volume, edited by leading systematists Joel Cracraft (AMNH) and Michael Donoghue (Yale University), begins with three chapters that explain why assembling the Tree is important to science and society. Most of the reasons offered will be familiar to biologists, as the revolution in systematics has penetrated many different fields. Unfortunately, nearly all refer to the benefits of knowing the phylogeny for a particular group and say little about those benefits that can only come from assembling the entire Tree. In addition to revealing common patterns or coordinated evolution among clades, having the whole Tree should lead to more important but as yet unanticipated insights. For example, would Wegener have imagined continental drift if he had only a collection of road maps and no global map to work with? We biologists need our own globe.

Following the introductory section, 26 chapters by authorities on major branches (clades) summarize the state of our phylogenetic knowledge. These begin at the base of the Tree, where contributors highlight, for example, the recent recognition that the earliest branchings split life into three domains: the bacteria, archaea, and eukaryotes. The chapters then proceed up the Tree through smaller branches and less inclusive groups (e.g., green plants, animals, and arthropods) to consider such "crown" groups as flowering plants, annelid worms, and birds. In each chapter, the authors summarize the constituent subgroups and typically describe supporting evidence, regions of uncertain relationship, and definitive morphological features. Afterward, Donoghue and three other leading evolutionary biologists (Edward Wilson, David Wake, and David Hillis) offer short summary perspectives. In the final chapter, the editors tie everything together by assembling a 138-taxon synoptic tree.

Taken individually, the chapters are useful summaries of our current understanding, but they seem like disconnected limbs. Nonetheless, the Tree will start to assemble itself—an emergent property of the disconnected parts—in the minds of those readers who take the time to read far enough. In that indirect way, the editors have met their goal. In addition, even the most broadly trained comparative biologists will discover unap-

Iconic metaphor.

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BROWSINGS

The Elements. Earth, Air, Fire, and Water. *Art Wolfe, text by Craig Childs.* Sasquatch, Seattle, WA, 2004. 176 pp. $45. ISBN 1-57061-405-9.

In ancient Greece, philosophers held that the physical world around them was derived from four elements: earth, air, fire, and water. Wolfe presents a collection of his color landscape and nature photographs that explores the diversity of forms these substances take. There are sheer rock walls of the Karakoram Range, Pakistan, and loose sediments of the Colorado River's subaerial delta plain, Baja California (left); morning mists and midday clouds; volcanic eruptions and flaming forests; Hawaiian breakers and Antarctic ice. The four elements are also used as background for portraits of wildlife, flowers, and trees. Many of the images are carefully composed to capture patterns of light and contrast. In four short essays, Childs offers his impressions of the effects the elements have on humans and the natural world.

preciated diversity in less familiar groups and the kind of fascinating organisms that inspired many of us to become biologists. These benefits would have been even easier to appreciate if the material was presented in the more dynamic and immersive experience of the volume's Web analog, the Tree of Life project (http://tolweb.org/tree/). (It is a shame that updatable, peer-reviewed Web pages still lack the professional status of static book chapters.)

Most authors have taken their charge very seriously and have written unbiased, synthetic, and useful accounts. Particularly readable chapters include those on Holometabola (insects characterized by complete metamorphosis), land plants, and chordates (vertebrates, hagfish, lancelets, and tunicates). A minority of the contributors have yielded to provincialism, focusing on their own work or dismissing information (e.g., molecular) that they distrust. The most extreme position appears in the mammal chapter, whose authors eschew the summary format in favor of lecturing on their preferred systematic procedures. Only a handful of conflicting conclusions appear; one is the description of the Holometabola as a group whose monophyly is either routinely supported by both morphology and molecules (Whiting) or never supported in any molecular data (Rainer Willmann).

The volume's principal utility stems from its revelation of the patterns among diverse clades. Many authors cite the explosion of molecular data as the reason for the revolution in phylogenetics, especially for the field's transformation since the previous symposium that attempted to view phylogenetics across all of life (*1*), held in 1988. The most publicized cases of conflict between molecules and morphology are not representative of that revolution: The tidal surge of molecular data seems to have confirmed numerous old hypotheses while rejecting a few but, most importantly, resolving many branches that morphological evidence did not. One is struck by the great reliance on a single gene—the small subunit (SSU) of the ribosomal DNA, also known as 18S—for most resolution deep in the Tree, even within phyla. Elsewhere, despite frequent accolades to molecular data, the recognition of many clades (especially among chordates) continues to rely on morphology.

The other broad impression the volume leaves is that of an imbalance toward authors who favor parsimony for phylogenetic analysis over model-based or statistical methods such as likelihood. Individually, this imbalance is not very important because all chapters include authoritative authors. The reviews of findings by other researchers are generally fair, although occasionally conflicting model-based results are brushed aside—as in the treatment of the debate over the effects of long-branch attraction on analyses of the relationship between the fly orders Strepsiptera and Diptera (*2*). A more pervasive and subtle, yet profound, consequence of this methodological bias is omission from most chapters of fundamental aspects of evolutionary history, like timing of events and rates of diversification. The emphasis in the volume is entirely on the sequence of branching. (Branch lengths are not important in parsimony analysis, and their estimates are generally unreliable. In contrast, they are integral to model-based methods.) As a result, the tempo and mode of evolution (*3*) are lost, and we cannot see whether the Tree looks like a spreading oak, a willow, or a bamboo grove—we have little sense of its gestalt. The lack of resolution in some parts of the Tree is therefore attributed to a lack of data rather than to the much more interesting possibility of rapid diversification. Branch lengths—as indicators of time or amount of evolution—are important to almost every aspect of comparative biology, and the volume would have benefited from the more nuanced vision their consideration would have offered.

The summary chapters praise the progress and promise more to come. I would have preferred a more critical analysis of the overall state of this resource-limited field. Where are the biggest holes? Should we focus on broad taxonomic coverage of a few universal genes, overlapping sets of many genes, or perhaps new initiatives to train morphologists? But in the end, the big picture emerges from the details, and we gain a better appreciation of how the branches fit together and where some of the bigger questions remain. The vision Cracraft and Donoghue articulate in their introduction does emerge from the mist, incomplete though it may appear. *Assembling the Tree of Life* should also meet the editors' larger goal. It will help the systematic community aspire toward a common goal, identify priorities for future coordinated work, and mobilize our resources.

References and Notes

1. B. Fernholm, K. Bremer, H. Jörnvall, Eds., *The Hierarchy of Life: Molecules and Morphology in Phylogenetic Analysis (Nobel Symposium 70,* Elsevier, Amsterdam, 1989).
2. J. P. Huelsenbeck, *Syst. Biol.* **46**, 69 (1997).
3. G. G. Simpson, *Tempo and Mode in Evolution* (Columbia Univ. Press, New York, 1944).

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CREDIT: ART WOLFE FROM *THE ELEMENTS*

It Takes a Village: Medical Research and Ethics in Mali

Ogobara K. Doumbo

The world is populated by rich and poor nations filled with a diversity of peoples and customs. For those scientists and physicians trying to have an impact on global health, conducting clinical trials and other types of medical research in these varied places, and among these different cultures, is central to the cause. It also is imperative that these efforts are undertaken in ethical ways that respect and honor those many individuals and communities that agree to participate in the investigations. To do that, researchers need to develop an understanding of the unique ways in which different cultural groups make decisions. Only then can the investigators feel assured that the human beings who consent to partake in their studies are fully informed about the possible risks and benefits of such participation.

This yearlong essay series celebrates 125 years of *Science* by inviting researchers from around the world to provide a regional view of the scientific enterprise. Series editor, Ivan Amato

Without knowing it at the time, I began to develop a sensibility to these issues as an 8-year-old child when I used to follow my grandfather while he practiced traditional medicine in the Dogon country in northeast Mali. I was impressed by his attitude toward his patients. He was very close to them, talked to them with respect and consideration, and showed compassion for their suffering. The patient and the caregiver in this care-providing system are so intimately connected that my grandfather considered the patients as part of his family, sharing the same food and shelter. Because of my grandfather's fame, and because he specialized in two diseases—pharyngitis and breast tumor/infection—some patients came to him from very remote areas.

The respect my grandfather showed while interacting with his patients marked my life. At the age of 10, I decided to become a medical doctor, and 7 years later I began my medical studies at the National School of Medicine and Pharmacy in Bamako, Mali. There, in my second year of medical studies, during rounds with the late Bernard Duflo of the Internal Medicine ward of the National hospital "Point G," I witnessed a similar intensely ethical attitude in the patient-physician relationship. My grandfather and Dr. Duflo, a French physician, were from two completely different cultures, but they shared the same attitude in healing patients.

My "indoctrination" in the importance of ethics continued when I started my own medical research career in 1984. I was fortunate at that time to have been at the University of Marseille where I worked with parasitologist Philippe Ranque in the first Phase II trials in Mali of ivermectin, then a new drug for the treatment of onchocerciasis, or river blindness. Like my grandfather, Dr. Ranque's priority was to show his patients attention, care, and compassion. Dr. Ranque believed that protection of volunteers in medical trials was more important than achieving scientific goals.

Over time, my exposure to this sort of ethics-in-the field, as well as to more academic discussions of ethics, convinced me that scientists must consider ethical issues throughout the entire research process, from identifying a research question to analyzing and interpreting data.

In Mali, where most of the population cannot read and where the social structures demand that decision-making be done more communally than in the West, we have had to develop new procedures for obtaining informed consent from participants in our malaria research. The process doesn't always work, takes a long time, and requires more discussion and leg work than in the West, but it generates mutual understand-

CREDIT: OGOBARA DOUMBO

Ogobara K. Doumbo
Mali

Ogobara K. Doumbo, director of the Malaria Research and Training Center at the University of Bamako, knew he would become a doctor at the age of 10. Immersed initially in the traditional medicine of his grandfather, he earned his medical credential in his home country and then later earned a Ph.D. in tropical medicine and parasitology at the Universities of Marseille and Montpellier in France. With expertise also in tropical medicine and parasitology, and with extensive research connections throughout the world, Dr. Doumbo has been making a global impact on the study, treatment, and prevention of malaria. With both in-the-field and academic exposure to medical ethics issues, he also has been helping to develop research protocols that respect and honor the diverse populations that become involved in his medical research. In 2003, following a nomination by the president of Mali, Dr. Doumbo became a member of his country's National Committee on Ethics. He also has been knighted by France's Legion of Honor and Mali's National Order. His center was selected in 2003 as a Center of Excellence in Clinical Research by the Agence Universitaire de la Francophonie (AUF) and as a malaria vaccine site by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health in Bethesda, Maryland.

All essays appearing in this series can be found online at www.sciencemag.org/sciext/globalvoices/

"Wabu" is a term that traditional healers in Mali use when referring to a condition marked by fever, seizures, delusions, or coma, the hallmarks of severe malaria. Traditional thought assigned the cause of wabu to a bird taking the spirit of a child at the moment that the bird and the child cry out simultaneously.

ing, medical care for the participants and often their entire communities, and valuable scientific results.*

Ethics in the Field: Informed Consent

At the Malaria Research and Training Center (MRTC) of the University of Bamako, Mali, we conduct different types of research, including laboratory-based studies, field research, and clinical trials. For us, ethical considerations constitute a dynamic process and are an integral part of the research enterprise. Here, I will focus on some study designs used at the MRTC and how we incorporate ethical considerations in our procedures for obtaining informed consent. I will also compare the strategies for getting documented and signed informed consent in developing countries, on the one hand, and in North America or Europe, on the other.

In recent years, we conducted an observational study of the natural history of malaria in the field as part of the Mali-Tulane Tropical Medicine Research Center (TMRC) project, funded by the National Institute of Allergy and Infectious Diseases (NIAID). The grant included three projects all located at Bancoumana, a large rural village in Mali with 12,000 inhabitants.

In Project 1 we studied a cohort of about 3500 children under 9 years of age, all of whom had been exposed to malaria. We recorded data on the natural history of malaria with the aim of identifying risk factors for infection or disease.

Because our studies were being done in the field and in a variety of social structures that respond differently to medical researchers coming into their lives, we needed to develop a dynamic approach to obtaining informed consent and to maintaining it over time. Usually, this was a stepwise process. First, we needed to get permission from the community to proceed. This began as a discussion with the group of village elders. Next, we convened focused group discussions with the heads of extended families. Then we held similar discussions with mothers whose children might become part of the malaria study. Finally, we obtained consent of the individual families involved in our cohort. The consent process was open and better suited to the needs of the population than were more conventional approaches. It generated more confidence by the villagers in the research project and a better understanding for us of the village culture and behavior. In developing this approach, I always had my grandfather and other ethics mentors in mind.

The villagers in the study received a tangible benefit too. Because we needed to examine the children many times during the course of the study, it meant that the villagers benefited from repeated contacts with the study physicians who lived in the village. Previous malaria studies in Africa commonly used a design typical of developing countries in which the interaction between study subjects and investigators is more limited and usually confined to clinical settings.

The second TMRC project was also unique in the strategy of getting informed consent. We wanted to test gametocyte infectivity in the community by directly exposing members already carrying the gametocytes to *Anopheles gambiae* mosquitoes—the most effective vector of the malaria parasite, *Plasmodium falciparum*, in Mali—collected in that village. Our aim was to determine the factors that influence how efficiently the mosquitoes would pick up the gametocytes from the infected villagers to initiate the sporogonic cycle during which the infective form of the malaria parasite proliferates inside the insects. Such direct exposure to these mosquitoes—even ones that were themselves not yet carrying the parasite and grown under controlled conditions—generates many ethical concerns. How could we prove to ourselves and to the community that the lab-reared *Anopheles* really were safe? How should we select the volunteers? How should we approach the community and document the process of community permission and individual consent (versus assent)? How should we explain the malaria life cycle to a population where 70 to 80% of its members are illiterate?

The first time we presented our protocol to the Institutional Review Board (IRB) of the Faculty of Medicine, Pharmacy and Odontostomatology, which oversees informed consent procedures, and to the village community, the design was rejected. The IRB deemed that it was not acceptable to expose humans to *Anopheles* even if the insects were reared in a well-qualified laboratory. We had to spend days and nights explaining the protocol to the villagers and to the Malian IRB members. Part of the process involved bringing representatives from two villages to visit our center at Bamako so that they could see how the exposure procedure would unfold. They spent 2 days with us and questioned us about all aspects of the experiment. In the end, the protocol was accepted, providing testimony to the ways in which responsible, open, and patient explanation of well-designed studies sometimes can alleviate participants' fears and misgivings.†

The third NIAID-funded project—in collaboration with colleagues at the U.S. Centers for Disease Control in Atlanta, GA, and the National Malaria Control Program of Mali—was designed as a supplement to the original TMRC project. It was particularly ethically charged because it involved a vulnerable population of pregnant women. We wanted to conduct this study to generate data important for developing public health policies to help protect pregnant women from contracting malaria in ways that minimize risks to the fetus.‡

We designed a community-based open label trial to compare three prophylactic drug regimens in pregnant women. Given the special importance of

Champion Infector: The *Anopheles gambiae* mosquito is the most effective vector of malaria in Mali.

CREDITS: (TOP TO BOTTOM) PAUL A SOUDERS/CORBIS; WORLD HEALTH ORGANIZATION

pregnant women in African culture, we knew that we needed to be extremely careful about ethical considerations. Although these drugs were recommended by the National Malaria Control Program and the World Health Organization, their use in pregnant women needed rigorous ethical scrutiny.

Central to the project, of course, was to obtain the community's permission to conduct the study. A key early step was to discuss the benefit of learning more about these drugs with the women's local council. Then we met with the mothers-in-law of each pregnant woman. After all of these discussions, we met with the individual pregnant woman. Toward the end of the process, the women requested that we meet with their husbands and fathers-in-law as well. (It is a widespread custom in African cultures that a pregnant woman belongs to the family-in-law, which is responsible for her care.) The overall goal of this project was to determine risk factors for contracting malaria, and we were able to offer the community the benefit of yearly feedback of all the results about the likely infectivity of their local mosquito population, before the next transmission season.

Unconsenting hosts. Women of child-bearing age, like these three walking to market in the Dogon region of Mali, are particularly vulnerable to malaria.

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Consenting Partners

These three experiences show how different the informed consent process needs to be in different places in the world. For one thing, the issue of who "signs off" on consent must be carefully considered. Informed consent in Europe and in North America usually involves written documents, which the prospective volunteer must read and sign in order to participate in a study. The emphasis is on the autonomy of individuals.

In some developing countries, however, individual and community consent are part of the same process. We cannot separate them in our countries, and this reality should be understood by sponsors and funding agencies and northern research institutions. We need to think about the protection, safety, risks, and benefits of individuals and of the community at large. We have learned in particular that the initial focus and discussion should be with the leaders of the community, rather than individuals. By approaching individuals first, one is likely to introduce social conflict in the village, and this could be unethical.

Another challenge is the need to document the consent process using a signed document. At the beginning of our TMRC projects, the villagers we approached were opposed to signing any document, because they strongly believed that "they gave their words" and that that should be sufficient. It took very careful explanation and patience to overcome this resistance. Informed consent must be based on a thorough understanding of the society in which the study is to take place. For outsiders, the role of local guides, local investigators, and socio-anthropologists is critical.

Documents and legal language also pose difficulties. One of them is that in developed countries, the heavy use of legal language and documents makes it increasingly difficult and murky for participants to discern the risks and benefits of participating in studies. The goal of these legal documents seems to have more to do with protecting the investigators and sponsors than the volunteers. What's more, legal language is hard enough for a highly educated person to understand. In Mali, less than 20% of adults are literate, so written documents can easily discourage rural populations from participating in studies. Also, for rural communities, paper often means trouble with the government.

From both ethical and biological standpoints, the particular case of pregnant women needs special attention. In Malian traditional communities there is a very good but discreet representation of women in decisions concerning the village's affairs. Foreign researchers may overlook this important involvement of women and thereby lose the confidence of the community. Similar decision-making subtleties go with enrolling children in medical trials.

In the developed countries, children normally are excluded from medical trials of new medicines, but in Africa we have to test products on children because the target populations for malaria and many infectious diseases are children and pregnant women. It can be especially challenging to convince the community that it ultimately is in its best interest to allow children to participate in trials that could have widespread medical benefits in the future.

One way to earn the confidence of the community is to provide medical care for the community while the study is being conducted. In rural regions of developing countries where medical care is limited or nonexistent, the research team often has to set up its own clinic. Providing standard care for both study participants and others in the community during a research project where the team is the sole source of medical care can be a form of community compensation. Almost all studies in developing countries should guarantee care for volunteers who experience serious adverse events during the study and after it has been completed. Care must be taken to ensure that the provision of these services, and the establishment of a clinic, do not induce the community to participate in the study when in fact it might not be in the community's interest to do so. This ethical dilemma is not yet solved and remains a big concern.

The challenge of obtaining informed consent for medical research from communities in developing countries can be daunting, especially from rural and mostly illiterate populations in rural regions. Even so, our 15 years of international research experience in dealing with these complex issues of informed consent in Mali shows that these difficulties can be overcome in a way that benefits medical science and public health.

Notes

*O. Doumbo, Multilateral Initiative on Malaria (MIM), Durban, South Africa, March 1999.
†Y. T. Touré *et al.*, *Am. J. Trop. Med. Hyg.* **59**, 481 (1998).
‡K. Kayentao *et al.*, *J. Infect. Dis.* **191**, 109 (2005).

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OCEAN SCIENCE

The Ocean's Seismic Hum

Sharon Kedar and Frank H. Webb

The first seismometers capable of measuring ground vibrations with periods of several seconds were installed in the early 20th century. Since then, the devices have recorded a continuous seismic hum, called "ocean microseisms." This hum is not the result of tectonic forces, but rather the response of the solid Earth to ocean wave-wave interactions, which have an annual global cumulative seismic energy comparable to that from earthquakes.

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Long considered noise by seismologists, ocean microseisms have recently been found to be a useful resource for the interdisciplinary study of our planet. They provide a record of the state of the oceans since the early 20th century and are a passive seismic source for probing the geological structure of Earth's upper crust (0 to 20 km). Origins, applications, and future studies of ocean microseisms were discussed at the Fall 2004 meeting of the American Geophysical Union (*1*).

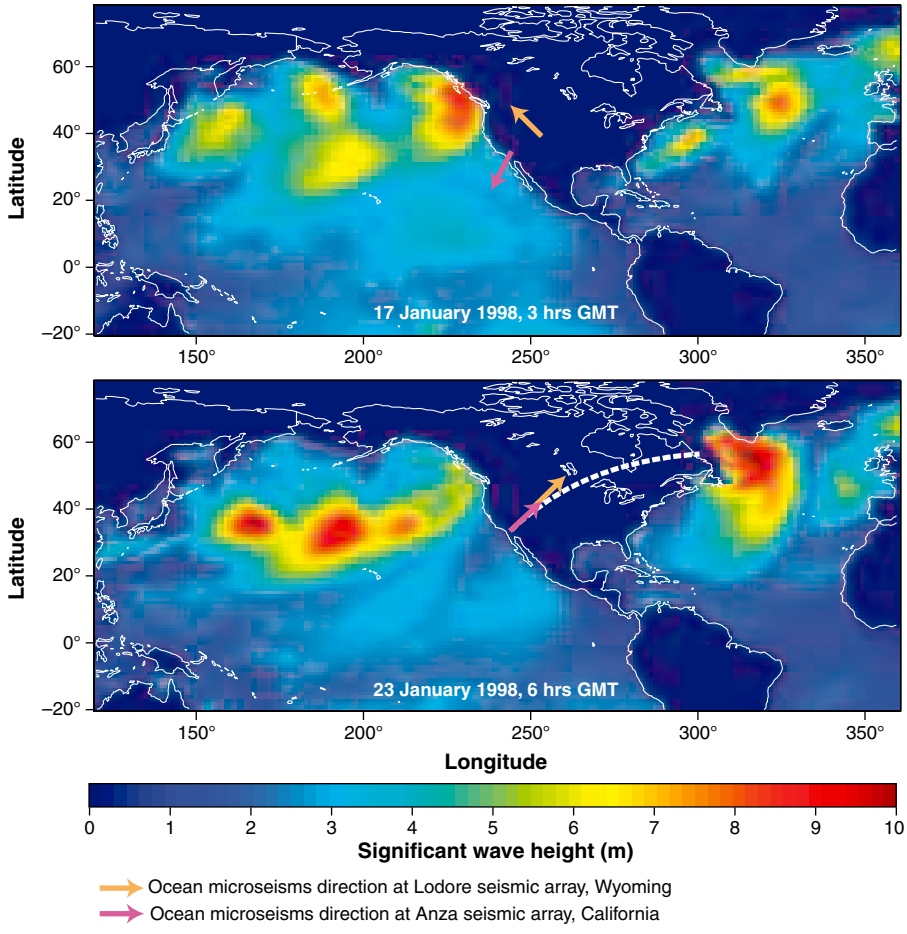
Early studies showed that ocean microseism signals are linked to ocean swell conditions (such as wave direction, amplitude, and period) but have half the swell period. The debate over their origins was settled by Longuet-Higgins, who showed in 1950 that the interaction of water waves with similar frequencies but opposing directions generates a second-order pressure wave with half the period and an amplitude proportional to the product of the wave heights (*2*). Unlike the pressure field generated by traveling waves, this pressure wave does not wane with depth and efficiently couples with the solid Earth to generate seismic surface waves.

The conditions for generating these wave-wave interactions probably occur at cyclonic depressions and along steep coastlines. Until recently, information about the source of ocean microseisms was inferred from laboratory experiments, analysis of seismic data, and sporadic ocean observations from buoys and ocean-bottom pressure sensors (*3*–*5*). In recent years, the combination of seismic data and improved global ocean-wave observations and models has led to new insights into the origins of ocean microseisms and new Earth science applications of these signals.

Potential oceanographic applications were considered as early as the 1960s, when Haubrich *et al.* showed that storms can be located and tracked with seismic data (*4*). This method has been improved upon in recent years. Bromirski and Duennbier (*6*) have shown that the wave climate (that is, the ocean conditions that give rise to and sustain ocean waves) can be reliably reconstructed with archived seismic data. This approach allows, for example, the strengths of El Niño conditions to be assessed for times when ocean data were largely unavailable.

Other researchers are combining remote sensing data from ocean satellite altimeters and data from the global seismic network to investigate global interactions between the oceans and the solid Earth. Rhie and Romanowicz (*7*) have discovered that the ocean's hum extends to periods of several minutes and have shown that background vibrations of the solid Earth are excited in the northern Pacific ocean in Northern Hemisphere winter, and in southern oceans in the summer. Ocean microseisms thus act as a meter by which global-scale ocean activity can be monitored.

Microseisms are also used to probe Earth's structure through seismic tomography. Crustal-scale tomography typically



Tracking wave-wave interactions. The maps show global wave heights [from the NOAA Wave Watch III model (*12*), see color scale at bottom] and arrival directions of ocean microseisms at U.S. seismic arrays (from seismic data; colored arrows). (**Top**) Microseisms recorded in Wyoming are dominated by wave-wave interactions near the British Columbia coast, and those recorded in southern California by interactions off the coast of Baja California. (**Bottom**) A North Atlantic storm swell hitting the steep Labrador coast triggers transcontinental microseisms.

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employs energetic, sporadic sources such as large explosions or earthquakes. Because ocean microseisms are almost continuous, they offer the possibility to monitor temporal changes in the crust (induced, for example, by tectonic stresses and fluid flow) on much shorter time scales than is possible with sporadic sources. Suggested in 1964 (*8*), this method has become a reality with the proliferation of digital seismic data. At the meeting, Stehly *et al.* (*9*) showed that ocean microseisms can be used reliably for tomographic imaging.

To better understand and use this ocean–solid Earth interaction, better knowledge of the state of the ocean wave field that generates the ocean microseisms is required. Combining seismic observations and wave action models, Schulte-Pelkum *et al.* (*10*) have analyzed oceanic source regions and conditions for ocean microseism generation (see the figure). Typically, ocean microseisms are generated locally in coastal regions. However, several times a year, cross-continental seismic waves are excited when swell is reflected off a coast that is prone to ocean microseism generation (*10*). Rodriguez *et al.* (*11*) have proposed that microseism-generating regions both in the open ocean and in coastal regions should be directly observable and identifiable from space- and airborne instruments. They pointed to synthetic aperture radar as the prime candidate for identifying regions of interaction between swells of similar periods and opposing directions, which are necessary for the excitation of ocean microseisms.

Ocean wave-wave interactions and the seismic energy they generate have largely been forgotten by oceanographers and swept aside as noise by seismologists. The emerging interdisciplinary effort of the two communities to understand and use this fundamental interaction, taking advantage of the riches of modern ground- and space-based measurements, is promising. This joint effort is likely to benefit ocean and solid Earth science alike.

References

1. *Ocean Microseisms: Observations and Applications*, American Geophysical Union Fall 2004 meeting, 13 to 17 December 2004, San Francisco.
2. M. S. Longuet-Higgins, *Philos. Trans. R. Soc. London Ser. A* **243**, 1 (1950).
3. R. I. B. Cooper, M. S. Longuet-Higgins, *Proc. R. Soc. London Ser. A* **206**, 424 (1955).
4. R. A. Haubrich, W. H. Munk, F. E. Snodgrass, *Bull. Seismol. Soc. Am.* **53**, 27 (1963).
5. K. Hasselmann, *Rev. Geophys.* **1**, 177 (1963).
6. P. D. Bromirski, F. K. Duennebier, *J. Geophys. Res.* **107**, 2166 (2002).
7. J. Rhie, B. Romanowicz, *Nature* **431**, 552 (2004).
8. M. N. Toksoz, *Geophysics* **29**, 154 (1964).
9. L. Stehly, N. M. Shapiro, M. Campillo, M. H. Ritzwoller, Fall AGU 2004, abstract S13C-1072.
10. V. Schulte-Pelkum, P. S. Earle, F. L. Vernon, *Geochem. Geophys. Geosyst.* **5**, 10.1029/2003GC000520 (2004).
11. E. Rodriguez, S. Kedar, Fall AGU 2004, abstract S11C-04.
12. H. L. Tolman, User manual and system documentation of WAVEWATCH III, version 1.18, Tech. Rep. Ocean Modeling Branch Contribution 166, MOAA/NWS/NCEP, 1999.

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GENETICS

A Century of Corn Selection

William G. Hill

Using conventional selection methods, plant and animal breeders have made many beneficial changes to the yields and composition of crops and livestock (*1*). Yet we know little about the numbers, effects, and mode of action of the genes that account for these long-term changes. A recent paper about maize selection in the journal *Genetics* demonstrates that such information is slowly becoming available (*2*).

Since 1896, in one of the longest experiments ever, biologists at the University of Illinois have continuously selected maize (corn) to change the oil composition of its kernels (*1*, *3*). Separate maize lines have been selected for more than 100 generations according to whether the kernels contain high or low amounts of oil, a trait of agronomic importance (*3*). Typically, mean oil concentration was estimated in 60 or so ears (cobs) of maize, and seeds from only 12 were selected to propagate the next generation. The change in oil concentration was almost continuous (*3*) and substantial: From a base of about 5%, the high oil-producing line now has about 20% oil in the kernel, and the low oil-producing line has almost none (see fig. S1). The two maize lines differ by about 32 standard deviations (SD, 0.42% in the base population). Divergent selection in separate lines for kernel protein concentration gave similar responses, except that the low line reached a plateau at about 5% protein (*3*).

To explain the large response in terms of changes at the level of individual gene loci, Laurie, Dudley, and colleagues from the Monsanto Company and the University of Illinois recently reported an analysis of the maize data that itself took much time and work (*2*). Their goal was to identify quantitative trait loci (QTLs)—regions of the genome where genes influence the trait—by testing the association between markers and the trait, a standard technique of QTL analysis (*4*).

A cross between high and low oil-producing maize lines from generation 70 was randomly bred for 10 generations in a large population (*2*) to reduce the effects of linkage disequilibrium. From each of 500 inbred lines subsequently derived by self-pollination, DNA was extracted for genetic analysis and oil concentration was estimated in the ears of inbred plants and of hybrid plants obtained by outcrossing. The single-nucleotide polymorphisms (SNPs) chosen as markers of whether a region of the genome came from either the high or low oil-content line differed substantially in allele frequency between the lines or, exceptionally, filled gaps in the genetic map. After eliminating markers with strong linkage disequilibrium, the investigators focused their analysis on 440 SNPs. Because markers more than 20 cM apart were essentially in linkage equilibrium, real associations would be expected only between close markers and the trait of interest.

An analysis of variance that fitted each marker locus individually and in pairs in separate analyses of inbred and hybrid data showed that both dominance within loci and epistatic interactions between pairs of loci were weak relative to additive effects. This means that the oil concentration in heterozygotes was intermediate between that of the two homozygotes, and that effects at different gene loci did not interact. The high correlation (0.75) of QTL effects on oil content estimated from inbred and hybrid plants is a further indication that they act additively. With the use of a stepwise multiple regression analysis to select markers linked to QTLs and to account for linkage disequilibrium between them, 50 markers were selected for the inbred data and 39 for the hybrids (where differences are smaller). Significant effects were found on all 10 chromosomes, with some clustering in the genome.

A major problem in QTL analyses comprising many tests of significance is to compromise between declaring false associations while missing real ones. To assess their findings, Laurie *et al.* simulated data with effects distributed similarly to what they observed, and subjected these data to the same analysis. They concluded that they had detected about 63% of the QTLs and that about 33% of markers selected were not QTLs. Consequently, they calculated the correct number of QTLs to be about 50.

The estimated effects of the QTLs on oil concentration were all much less than the line divergence of 15% at generation 70. Indeed, the largest had an effect (half homozygote

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difference) of about 0.3% oil, and most had a difference of 0.1 to 0.2%. Only about 80% of the QTLs had effects in the same direction as the selection response (38 of 50 inbreds, 33 of 39 hybrids). For the others, the marker allele from the high oil-producing line was associated with low oil—however, alleles of small effect can be fixed by chance in the opposite direction to that of selection if few parents are selected (*5*). The detected QTLs, if segregating independently, could account for about half the genetic variance of the trait in the population and, by summing their effects, about half the divergence between the high and low oil-producing maize lines. The remaining QTLs are likely to have similar or smaller effects on oil concentration because those with large effects were unlikely to be missed given the degree of genome coverage and the size of the experiment.

Similar findings have also been obtained in a recent study on divergent selection lines of poultry. Andersson and colleagues (*6*) analyzed a large intercross of poultry lines that had been selected by Siegel in Virginia (*7*) for high and low body weight (at 8 weeks of age) for 40 generations and that differed in body weight by a factor of about 9. Although 13 QTLs were detected, none individually accounted for more than 3% of the body-weight variance in the F_2 generation, and each of these QTLs contributed only a small part of the divergence between the selected lines. Furthermore, the QTLs mainly had additive effects on body weight, as Laurie and colleagues found in their maize analysis.

In most other studies, however, QTLs of substantial effect have been detected— for example, QTLs for body size in poultry, not only in broiler × layer (*8*) crosses, but also in commercial broiler populations still segregating under intense selection (*9*). Some QTLs exerting large effects on the trait of interest found in mapping experiments have subsequently been identified as a single causative mutation—such is the case with the mutation in the gene encoding insulin growth factor–2 (IGF-2) in the pig, which alters muscle growth in these animals (*10*). Although such effects are real, effects of QTLs declared significant tend to be biased upward, and those of small effect are more likely to be missed (*4*). Models of the underlying distribution of gene effects indicate an exponential form, with numbers increasing as effects get smaller (*11*). Too much variation is therefore usually attributed to QTLs of large effect.

The recent studies of selected maize and broiler lines (*3*, *6*) were extensive, and the QTL effects identified were small. These appear to conform to the infinitesimal model of genes of small effect assumed in much quantitative genetic theory (*4*), which predicts the observed continuous steady responses to artificial selection. It is moot as to what defines a "small" effect, however. A maize line containing 0.2% oil in the kernel represents a difference of almost 1 SD between homozygotes in the maize base population; the largest effects detected in the chickens were almost as big (the variance in the F_2 was much higher than in the base). The continuing responses to selection, therefore, are not likely to be due mainly to continuing tiny changes in gene frequency predicted by the infinitesimal model; instead they may be due to the fixation of genes, including those arising by mutation after selection started (*12*, *13*), which have appreciable effects while segregating. The biological processes leading to oil concentration or chicken growth are obviously highly interactive, but genes that contribute to selection response must differ in effect when averaged over all other segregating genes. This may explain the Laurie *et al.* finding that their detected QTLs had approximately additive effects on oil production in maize. We have yet to discover how such QTLs work, but several of the SNPs associated with oil concentration were at candidate loci (*2*), so there are opportunities to find out. It is a challenge for geneticists to identify the genes and the molecular changes in them that cause these many small but important differences in quantitative traits. It is these small differences that generate variability in populations, providing fuel for change through the action of natural and artificial selection.

References

1. J. Janick, Ed., *Plant Breeding Reviews, Volume 24, Part 1: Long-Term Selection: Maize. Part 2: Long-Term Selection: Crops, Animals, and Bacteria* (Wiley, Hoboken, NJ (2003).
2. C. C. Laurie *et al.*, *Genetics* **168**, 2141 (2004).
3. J. W. Dudley, R. J. Lambert, *Plant Breed. Rev.* **24** (part 1), 79 (2004).
4. M. Lynch, B. Walsh, *Introduction to Quantitative Genetics* (Sinauer, Sunderland, MA, 1998).
5. A. Robertson, *Proc. R. Soc. London Ser. B* **153**, 234 (1960).
6. L. Andersson, P. B. Siegel, personal communication.
7. E. A. Dunnington, P. B. Siegel, *Poultry Sci.* **75**, 1168 (1996)
8. A. Sewalem *et al.*, *Poultry Sci.* **81**, 1775 (2002).
9. D. J. deKoening *et al.*, *Genet. Res.* **83**, 211 (2004).
10. S. Kerje *et al.*, *Anim. Genet.* **34**, 264 (2003).
11. B. Hayes, M. E. Goddard, *Genet. Sel. Evol.* **33**, 209 (2001).
12. W. G. Hill, *Genet. Res.* **40**, 255 (1982).
13. B. Walsh, *Plant Breed. Rev.* **24** (part 1), 177 (2004).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/307/5710/683/DC1
Fig. S1

ECOLOGY

Untangling an Entangled Bank

David Storch, Pablo A. Marquet, Kevin J. Gaston

Biodiversity, the most conspicuous property of life, has fascinated generations of ecologists and evolutionary biologists. Part of this fascination arises from the fact that only a small fragment of this diversity has been described and catalogued, providing endless opportunities to speculate about the rest. Part arises from the sense that any regularities and general patterns in the biodiversity that we see today exist despite, or perhaps because of, the complexity of the processes and interactions that have driven the dynamics of biodiversity through time and space. In the *Origin of Species*, Charles Darwin made a specific appeal to this idea when he wrote his famous description of the complex ecology of a bank covered by dense vegetation: "It is interesting to contemplate an entangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent on each other in so complex a manner, have all been produced by laws acting around us" (*1*).

Some general patterns in contemporary biodiversity do exist (*2*), and some of them are surprisingly simple. Take the species-area relationship, for example. The number of species increases with the area sampled, often linearly on a log-log scale, suggesting scale invariance. Such invariance is a prime area of investigation among those interested in complex systems. Many phenomena—ranging from the frequency distribution of species extinctions to allometric relationships between body size and rates of various biological processes (*3*)—reveal scale invariance, suggesting that simple rules underlie the structure and function of ecosystems. Some recent discoveries have shed light on the processes that define quantitative patterns in biodiversity. Dissecting these patterns was the goal of an international workshop held recently in Prague and co-organized by the Santa Fe Institute and the Center for Theoretical Study at Charles University (*4*).

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One major challenge is to relate patterns of species richness to the spatial distribution of individual species. Scale invariance of the species-area relationship, for example, has led to the development of models relating this phenomenon to the fractal spatial distribution of individuals, which is characterized by similar spatial patterns over several scales of resolution (*5*, *6*). But two questions arise. First, do species really exhibit fractal distributions? Using sophisticated analytical techniques applied at different scales of observation, Jack Lennon (Macaulay Institute, Aberdeen) and Fangliang He (University of Alberta) concluded that some species really do, but others do not, and that the level of fractality is related in part to the rarity of the species. Second, it is not clear why species' distributions should be fractal. Arnošt Šizling (Center for Theoretical Study, Prague) reported that a distribution that is indistinguishable from a fractal distribution may emerge from random processes of spatial aggregation on several scales of resolution. These findings suggest that fractals are simply a useful and analytically tractable approximation of the complex spatial aggregation of individuals that is responsible for patterns of biodiversity.

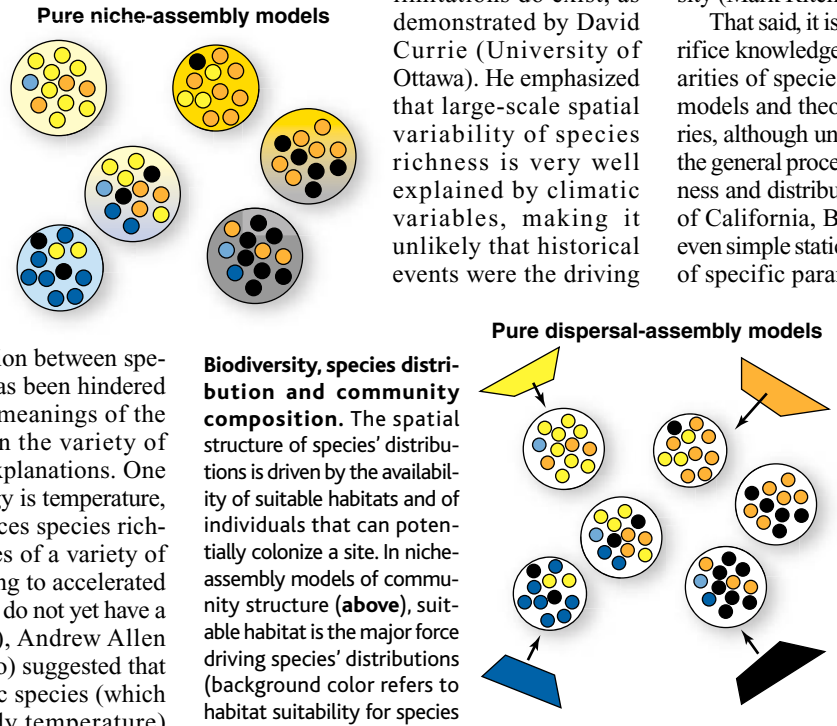
Another widespread observation is a positive relationship between species richness and available energy (*7*). Andrew Clarke (British Antarctic Survey, Cambridge) noted that understanding the connection between species richness and energy has been hindered by ignoring the different meanings of the word "energy" implied in the variety of mechanisms invoked as explanations. One important measure of energy is temperature, which presumably influences species richness by increasing the rates of a variety of biological processes, leading to accelerated speciation (*8*). Although we do not yet have a comprehensive theory (*9*), Andrew Allen (University of New Mexico) suggested that the richness of ectothermic species (which cannot regulate their body temperature) could be predicted from environmental temperature according to fundamental laws connecting body size, temperature, and metabolic rate. Another measure of energy is environmental productivity, which affects the amount of resources available to a population, enabling an increase in the numbers of individuals and the persistence of more species. David Storch (Center for Theoretical Study, Prague) showed how a simple model of spatial dynamics, which assumes that the probability of species occurrence is proportional to productivity, accurately predicts the increase in bird species richness with increasing environmental productivity. Thus, species richness is influenced by available energy in different ways. It is possible that the diversity of ectotherms may principally be driven by temperature, whereas resource availability may be more important for endothermic animals that are able to regulate their body temperature.

The extent to which external constraints limit biodiversity has long been a topic of debate. Typically, those models relating the species-area relationship to the distribution patterns of individual species ignore interspecific interactions or limits to the number of species or individuals that can co-occur in an area. But every ecosystem must have some finite capacity in terms of the number of individuals or biomass that it can sustain. James Brown (University of New Mexico) pointed out that this leads to a zero-sum situation that may affect biodiversity dynamics as well as species distribution. Relationships between environmental energy and biodiversity imply that such limitations do exist, as demonstrated by David Currie (University of Ottawa). He emphasized that large-scale spatial variability of species richness is very well explained by climatic variables, making it unlikely that historical events were the driving force for the current distribution of biodiversity on Earth.

This "zero sum" rule has been assumed in neutral theories of biodiversity that attempt to explain major phenomena concerning species richness, abundance, and distribution in terms of stochastic population growth, migration, and speciation. The original neutral theory formulated by Stephen Hubbell (*10*) can be extended to incorporate realistic dispersal processes (Luis Borda-de-Água, University of Georgia) and can predict many biodiversity patterns—such as the relationship between the richness of native and alien species (Tomaš Herben, Charles University, Prague). However, current neutral models are insufficient to explain observed patterns of species' distributions. Jerome Chave (Université Paul Sabatier) reported that both stochastic spatial processes and environmental heterogeneity (see the figure) contribute to the species distribution patterns of neotropical trees. At least at small spatial scales, the way that species divide resources and habitat is crucial for understanding patterns of biodiversity (Mark Ritchie, Syracuse University).

That said, it is sometimes necessary to sacrifice knowledge about the biological peculiarities of species and communities to make models and theories tractable. Neutral theories, although unrealistic, provide insight into the general processes that govern species richness and distribution. John Harte (University of California, Berkeley) demonstrated that even simple static models without the addition of specific parameters could be useful. His model predicts a variety of patterns in the spatial distribution and abundance of plant species just on the basis of the frequency distribution of total species abundance and a simple probability rule that relates occupancy at one spatial scale to occupancy at smaller scales. Although its biological interpretation is unclear, this approach shows that different patterns are intrinsically linked to one another, and it is important to study these inevitable links, for they can provide the basis for understanding the dynamics of complex ecological networks (Neo Martinez, Pacific Ecoinformatics and Computational Ecology Lab, Berkeley).

As physicist Murray Gell-Mann (Santa Fe Institute) observed, it is interesting how little we actually know. Although sophisticated techniques of data analysis are now available and theory is developing rapidly, macroecology as an exact science is still in its infancy. Many patterns have been docu-



Biodiversity, species distribution and community composition. The spatial structure of species' distributions is driven by the availability of suitable habitats and of individuals that can potentially colonize a site. In niche-assembly models of community structure (**above**), suitable habitat is the major force driving species' distributions (background color refers to habitat suitability for species with respective colors). The same pattern of species' distributions and local community composition (**right**) can, however, be interpreted in terms of the spatial distribution of sources of potential colonists of different species (colored polynoms) without the need to consider habitat suitability. These "dispersal-assembly models" give a reasonable prediction of general properties of observed spatial species' distributions and community structure, but fail to predict details about the local species composition of communities. [Adapted with permission from a figure by Jerome Chave, Université Paul Sabatier]

mented using only limited data sets, and even some phenomena assumed to be well documented (albeit with a multitude of potential explanations) may look different when examined from another perspective. For some taxa (such as the bacteria or protists), we have only weak evidence about basic patterns (Brendan Bohannan, Stanford University; Jessica Green, University of California, Merced). Obtaining better data across multiple scales and bridging the gap between theory and observation is crucial to achieve a better understanding of quantitative patterns in biodiversity. However, one thing is certain: Darwin's entangled bank is far more entangled than even he thought.

References

1. C. Darwin, *On the Origin of Species by Means of Natural Selection* (John Murray, London, 1959).
2. K. J. Gaston, *Nature* **405**, 220 (2000).
3. J. H. Brown *et al.*, *Ecology* **85**, 1771 (2004).
4. Scaling Biodiversity, 19–23 October 2004, Prague, Czech Republic.
5. J. Harte *et al.*, *Science* **284**, 334 (1999).
6. A. L. Šizling, D. Storch, *Ecol. Lett.* **7**, 60 (2004).
7. D. Currie, *Am. Nat.* **137**, 27 (1991).
8. A. P. Allen *et al.*, *Science* **297**, 1545 (2002).
9. D. Storch, *Science* **299**, 346 (2003).
10. S. P. Hubbell, *The Unified Neutral Theory of Biodiversity and Biogeography* (Princeton Univ. Press, Princeton, NJ, 2001).

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ASTRONOMY

At the Heart of the Milky Way

T. Joseph W. Lazio and Theodore N. LaRosa

At a distance of just 25,000 light years (2.5×10^{20} m), the center of our galaxy, the Milky Way, provides the foundation for understanding phenomena in other galaxies. The central black hole (*1*) and regions of intense star formation in its vicinity can be probed at 100 times the resolution of even the nearest galaxies. Nonetheless, even the basic properties of a key component of the galactic center, its magnetic field, remain poorly understood.

Magnetic fields have the potential to transform, store, and explosively release energy, to transport angular momentum, and to confine high-energy plasmas into powerful jet flows. They are therefore central to astrophysical activity from stellar to galactic scales.

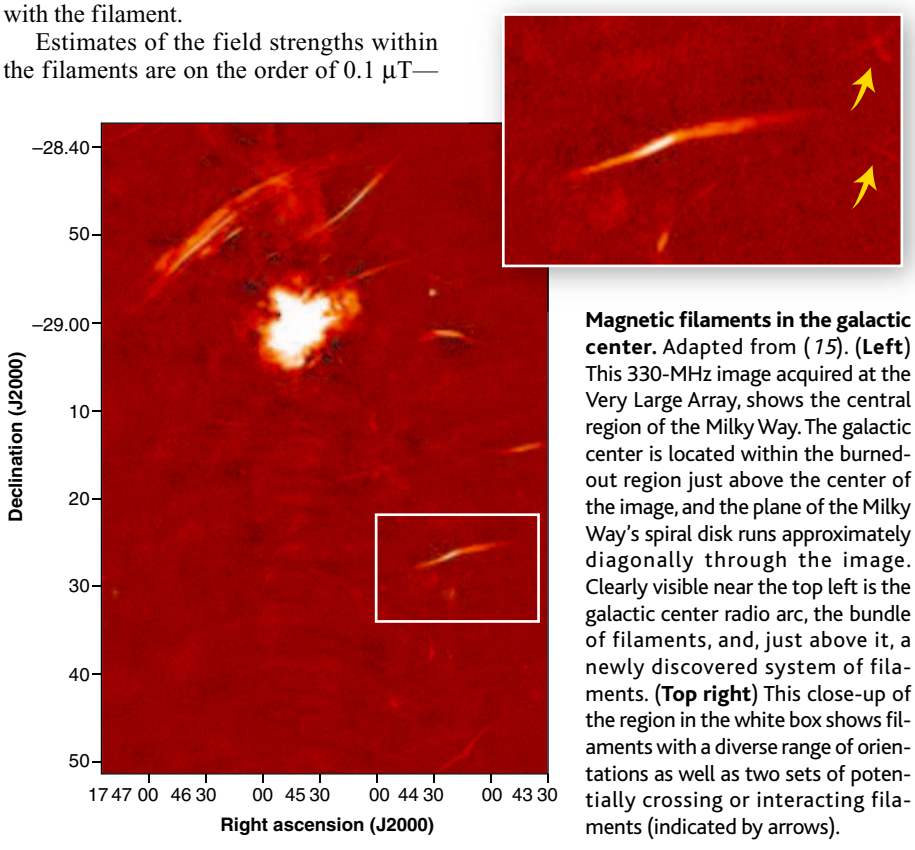
Magnetic fields are found throughout the Milky Way. Measurements suggest that the magnetic field in the spiral disk of our galaxy has two components, one globally ordered and the other random, with approximately equal strengths of ~0.3 nT (*2*); the globally ordered component generally follows the spiral arms of the galaxy. Key questions about the magnetic field in the galactic center are whether it is comparable in strength or much stronger than the field in the disk, and whether it is globally ordered or largely random.

About 20 years ago, the first high-resolution radio images of the galactic center (*3*) revealed numerous magnetic structures that are unique to the galactic center. The most striking of these is the galactic center radio arc, a series of parallel linear filaments, each of which is merely a few light years wide yet more than 100 light years long. Also observed were a number of isolated linear features that were variously referred to as streaks, threads, and filaments. The relation between these isolated filaments and the bundled filaments of the radio arc remains unknown.

These filamentary structures are distinguished by extreme length-to-width ratios (~10 to 100), nonthermal spectra, and a high intrinsic polarization (~30%, and in some cases approaching the theoretical maximum of 70% for synchrotron radiation). The polarization and nonthermal spectra are consistent with the filaments being produced by synchrotron radiation from relativistic electrons spiraling around a magnetic field. Detailed measurements of individual filaments have shown that the magnetic fields are aligned longitudinally with the filament.

Estimates of the field strengths within the filaments are on the order of 0.1 μT—nearly 1000 times the field strength in the galactic disk. In addition, the early studies (*4*) suggested that all filaments are essentially perpendicular to the galactic plane (within 20°). The picture that emerged was of a strong, dipolar magnetic field filling the galactic center. The filaments were explained as magnetic flux tubes, which were "lit up" by relativistic electrons that were accelerated by a local interaction such as magnetic field reconnection (*4*).

However, recent radio and submillimeter observations are challenging this simple picture. New wide-field images at radio wavelengths (between 20 and 90 cm, see the figure) have substantially increased the number of known filaments and have shown that the volume over which filaments occur is much larger than originally thought. With the larger number of filaments has come the discovery of filaments



Magnetic filaments in the galactic center. Adapted from (*15*). (**Left**) This 330-MHz image acquired at the Very Large Array, shows the central region of the Milky Way. The galactic center is located within the burned-out region just above the center of the image, and the plane of the Milky Way's spiral disk runs approximately diagonally through the image. Clearly visible near the top left is the galactic center radio arc, the bundle of filaments, and, just above it, a newly discovered system of filaments. (**Top right**) This close-up of the region in the white box shows filaments with a diverse range of orientations as well as two sets of potentially crossing or interacting filaments (indicated by arrows).

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that are not perpendicular to the galactic plane (*5*, *6*). A range of filament orientations (see the figure, top right panel) is difficult to reconcile with the idea of a strong, pervasive dipolar field. Moreover, the much larger volume over which filaments are found implies a much larger total energy contained in the magnetic field.

The diverse orientations and larger than expected volume for the filaments have prompted reconsideration of their origin. One possibility is that they represent localized, and potentially dynamic, enhancements of an otherwise weak magnetic field. For instance, in one model, the filaments are magnetic wakes generated when molecular clouds interact with a wind from the galactic center, analogous to cometary plasma tails formed in the solar wind (*7*). In other models, the filaments form when colliding stellar winds fill locally strong magnetic flux tubes. Of course, more than one model may be correct. The current models offer intriguing ideas for the origin of the filaments, but none can account for the full range of characteristics exhibitted by these structures. Clearly, their presence in the galactic center indicates that it is the only region in the Milky Way where they can form, or more generally, that only in the nuclei of galaxies are the conditions sufficient for filaments to form.

High-resolution submillimeter polarization observations have also recently provided insights into the magnetic field at the galactic center. In contrast to radio measurements, which probe the magnetic field in relatively dilute plasmas, submillimeter observations probe the thermal emission from dust grains in cold, dense clouds. If these dust grains rotate in a magnetic field, they will align perpendicular to it. The thermal emission from the grains is then polarized because of the grain orientation.

Large-scale observations show that the magnetic field in molecular clouds at the galactic center is aligned preferentially in a disk-like orientation, much like the magnetic field in the spiral disk (*8*). At higher resolution, the field traced by submillimeter polarization measurements is more complex, influenced at least in part by the local dynamics of the dust (*9*). In some regions, the field directions inferred from these measurements are similar to those found in the synchrotron-emitting plasma.

These observations at different wavelengths remain to be reconciled with each other and with suggestions that the filaments are also visible at x-ray wavelengths [see for example (*10*)]. New instruments under development should be able to detect many more filaments, determine their orientations and lengths, and assess the extent to which they are associated with other objects in the galactic center. These data may reveal whether the magnetic field in the galactic center is a result of a dynamo generated by the central black hole (*11*); a large-scale outflow driven by past injections of energy from the combined actions of many supernovae, the central black hole, or both (*12*, *13*); or even the amplification of a primordial field predating the formation the Milky Way (*14*).

References and Notes

1. G. C. Bower *et al.*, *Science* **304**, 704 (2004).
2. R. Beck, *Space Sci. Rev.* **99**, 243 (2001).
3. F. Yusef-Zadeh *et al.*, *Nature* **310**, 557 (1984).
4. M. Morris, E. Serabyn, *Annu. Rev. Astron. Astrophys.* **34**, 645 (1996).
5. C. C. Lang, K. R. Anantharamaiah, N. E. Kassim, T. J. W. Lazio, *Astrophys. J.* **521**, L41 (1999).
6. T. N. LaRosa, M. E. Nord, T. J. W. Lazio, N. E. Kassim, *Astrophys. J.* **607**, 302 (2004).
7. S. N. Shore, T. N. LaRosa, *Astrophys. J.* **521**, 587 (1999).
8. G. Novak *et al.*, *Astrophys. J.* **583**, L83 (2003).
9. D. T. Chuss *et al.*, *Astrophys. J.* **599**, 1116 (2003).
10. Q. D. Wang *et al.*, *Astrophys. J.* **581**, 1148 (2002).
11. S. K. Chakrabarti *et al.*, *Nature* **368**, 434 (1994).
12. R. A. Chevalier, *Astrophys. J.* **397**, L39 (1992).
13. J. Bland-Hawthorn, M. Cohen, *Astrophys. J.* **582**, 246 (2003).
14. B. D. G. Chandran *et al.*, *Astrophys. J.* **528**, 723 (2000).
15. M. E. Nord *et al.*, *Astron. J.* **128**, 1646 (2004).

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SIGNAL TRANSDUCTION

Signaling Specificity in Yeast

Elaine A. Elion, Maosong Qi, Weidong Chen

A central problem that continues to puzzle biologists is how cells translate myriad stimuli into highly specific responses. Signaling specificity is complicated in eukaryotic cells because signal transduction pathways that respond to different stimuli and perform distinct functions often share the same pathway components. In haploid budding yeast, for example, the mitogen-activated protein kinase (MAPK) cascades that regulate both mating and filamentous growth share the same three activating kinases—Ste20 MAPKKK or PAK-type kinase, Ste11 MAPKKK, and Ste7 MAPKK (which are activated serially)—as well as the Ste12 transcription factor (see the figure). Two separate MAPKs are activated by the same Ste7 MAPKK: Fus3, which is essential for the mating program, and Kss1, which is essential for the filamentous growth program (also called invasive growth in haploids and pseudohyphal development in diploids). Fus3 and Kss1 are both activated in response to mating pheromone by the MAPK scaffold protein Ste5; Kss1 also can be activated by cell surface proteins such as mucin (Msb2) and other conditions that promote invasive growth [(*1*–*3*); see (*4*) for a summary of targets in the mating and invasive growth pathway]. How is pathway specificity maintained with so much redundancy? Two papers in a recent issue of *Cell* reveal how the Fus3 and Kss1 MAPK signaling pathways are insulated from each other, ensuring smooth execution of the mating program without erroneous activation of the filamentous growth program (*5*, *6*).

Signaling specificity is possible in part because MAPKs choose different substrates. For example, during mating, Fus3 phosphorylates the cell cycle regulator Far1, whereas both Fus3 and Kss1 can phosphorylate Ste12 (see the figure). During filamentous growth, Kss1 regulates the Tec1 transcription factor indirectly through Ste12, which is guided by Tec1 to the promoters of filamentous growth genes (*4*). In addition, MAPKs use different activation mechanisms—for example, Fus3 must interact with the Ste5 scaffold to be activated, whereas Kss1 activation does not have this requirement (*1*–*3*). However, these levels of control do not explain how the kinases are insulated from erroneous cross-activation. For example, why doesn't activation of mating pheromone by Kss1 induce a filamentous growth program?

One level of insulation comes from attenuation of nonessential MAPKs under any given condition. During filamentous growth, selective repression of Fus3 by phosphatases such as Msg5 enforces the filamentous growth program (*1*). During pheromone stimulation, Fus3 attenuates the activation of Kss1 and influences its strength and duration of signaling (*7*). Another key level of insulation comes from the inhibition of filamentous growth by Fus3 and the expression of Tec1-dependent genes during pheromone signaling (*8*). The mysterious mechanism of this potent insulation is now revealed by the two *Cell* papers (*5*, *6*) and a third study (*9*). These reports show that the Fus3 and Kss1 signaling cascades are insulated from each other because Fus3 controls degradation of Tec1, the transcription factor essential for activation

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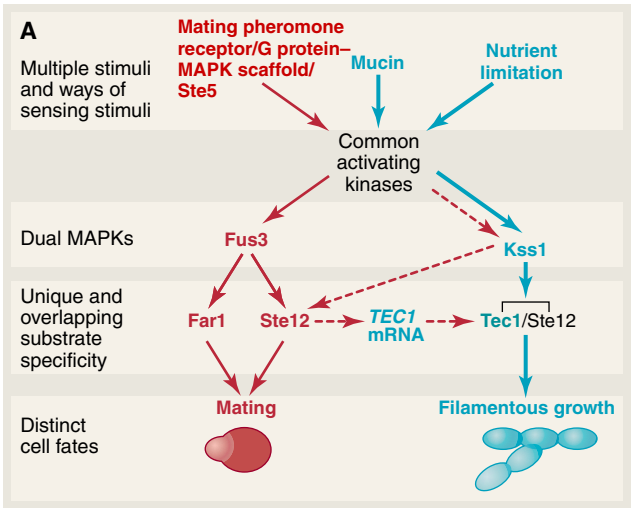

No fuss about insulation. (**A**) The Fus3 and Kss1 MAPKs are activated by the same signaling cascades—that is, they share the common activating kinases Ste20 MAPKKKK, Ste11 MAPKKK, and Ste7 MAPKK. Although Fus3 and Kss1 are both activated by mating-specific inputs that involve the Ste5 scaffold protein (red; pheromone receptor, G protein–MAPK scaffold), only Kss1 can be activated independently of Ste5 by inputs that promote filamentous growth (blue; the mucin Msb2 and nutrient deprivation). These two MAPKs have both common and unique substrates. Fus3 phosphorylates the Far1 cyclin-dependent kinase inhibitor and the Tec1/Ste12 transcription factor, two events critical for mating. Kss1 regulates the activation of Tec1/Ste12, which is necessary for filamentous growth. During mating, Kss1 activation switches on expression of both mating and filamentous growth genes, including the *TEC1* gene (depicted by dashed arrows as they are not essential for mating and interfere with cell cycle arrest, which is needed for terminal differentiation). (**B**) Fus3 phosphorylation of the Tec1 protein on Thr^{273} leads to ubiquitin-dependent degradation of Tec1 by a SCF ubiquitin-ligase complex, neatly blocking entry into the filamentation program. Thus, the status of the Fus3 MAPK determines whether cells mate or enter a filamentous growth program. This regulatory device is not necessary in diploid cells, which do not mate or express Fus3.

of the filamentous growth program (see the figure). The beauty of these studies is that they use established yeast mutants that are hyperfilamentous as a result of mutations in Tec1 (Thr^{273} switched to methionine, and Pro^{274} switched to serine). These yeast mutants point the way to discovering the critical residue in Tec1 that is phosphorylated by Fus3, signaling the degradation of Tec1. Thr^{273} and Pro^{274} together form part of a consensus phosphorylation site for proline-directed MAPKs (*5*, *6*). The threonine residue at position 273 is one of multiple sites phosphorylated by Fus3 in vitro (*5*, *6*, *9*) and in pheromone-treated cells in vivo (*5*). The proline residue at position 274 is predicted to be necessary for recognition of the threonine by a MAPK. However, Tec1 mutants with a methionine at residue 273 or a serine at residue 274 are resistant to pheromone-induced degradation and display almost the same absence of insulation as a yeast mutant that completely lacks the *FUS3* gene (*5*, *6*). These findings argue compellingly that down-regulation of the Tec1 protein is the major way in which Fus3 insulates the mating MAPK cascade from the filamentous growth MAPK cascade. In contrast, Kss1 does not phosphorylate Tec1 (*5*, *6*, *9*), ensuring that Tec1 remains stable during filamentous growth. Interestingly, the amount of Tec1 degraded is proportional to the concentration of the pheromone stimulus (*5*). This is consistent with earlier findings by several groups showing that a low concentration of mating pheromone induces production of *TEC1* mRNA and invasive growth. Thus, the system may have evolved to permit dividing yeast both to forage by filamentous growth and to search for optimal mating conditions while cells are primed for mating, and before Tec1 is degraded.

Additional evidence suggests that phosphorylated Tec1 may be recognized for destruction by ubiquitin-dependent proteolysis through the action of an SCF (Skp1-Cdc53/Cul1–F-box) ubiquitin ligase complex (*5*, *6*). Tec1 is ubiquitinated in the presence of mating pheromone (*5*, *6*) but not if residue 273 is mutated or Fus3 is absent (*6*). In addition, Tec1 is completely stabilized in a *cdc34-2* yeast mutant (which lacks the E2 enzyme) (*5*, *6*) and a *cdc53-1* mutant (*6*) in the presence of pheromone (although it is possible that these pleiotropic mutations could have interfered with the activation of Fus3). The Chou *et al.* (*5*) and Bao *et al.* (*6*) groups reach different conclusions about the identity of the F-box protein that recognizes phosphorylated Tec1 and links it to the SCF ubiquitin-ligase complex. Chou *et al.* (*5*) propose SCF^{Cdc4} (WD40 class) as their candidate for the following reasons. Tec1 is stable in a *cdc4-3* mutant although it is phosphorylated at residue 273; Fus3-phosphorylated Tec1 interacts with Cdc4 in vitro; and Tec1 cannot be ubiquitinated in a *cdc4-3* yeast mutant. In their turn, Bao *et al.* (*6*) propose an as yet undefined SCF^{Dia2} protein (LRR class by homology) on the basis of the stabilization of Tec1 in a *dia2* mutant that is known to be hyperfilamentous (*10*). Although it is possible that multiple SCF complexes regulate Tec1 [and the data in Chou *et al.* support short-term stabilization of Tec1 in a *dia2* mutant as well as in a *grr1* mutant that is also hyperfilamentous (*10*)], more work is needed to dissect the possible interplay between different SCF complexes and between the direct and indirect effects of mutations on Tec1 production.

The most important notion gleaned from these studies is that pathway specificity can be regulated by the MAPKs downstream of the MAPK signaling cascades. Previous work supports the possibility that MAPK-induced proteolysis of MAPK targets may be a general means of controlling pathway specificity (*11*, *12*). Such a regulatory device permits flexibility by focusing control on specific MAPK outputs while maintaining others that may be beneficial for the desired program. Proteolysis is advantageous because it is rapid and irreversible, allowing for sharp transitions. Phosphorylation-induced proteolysis of substrates provides a way to get rid of unwanted by-products of activated protein kinases at sites distal from the place of activation. There are additional opportunities for regulating substrate: at the place and time of substrate degradation, and during various phosphorylation events that are required for substrate to be recognized by the proteolysis machinery. These different methods of regulation are also applicable to feedback and cross-regulation of signal transduction pathways. Perhaps Nature has found a simple strategy to take full advantage of redundancies in a system that has been designed to be exquisitely sensitive.

References

1. J. Andersson *et al.*, *EMBO J.* **23**, 2564 (2004).
2. S. Maleri *et al.*, *Mol. Cell. Biol.* **24**, 9221 (2004).
3. P. J. Cullen *et al.*, *Genes Dev.* **18**, 1695 (2004).
4. J. Zeitleiger *et al.*, *Cell* **113**, 395 (2003).
5. S. Chou *et al.*, *Cell* **119**, 981 (2004).
6. M. Z. Bao *et al.*, *Cell* **119**, 991 (2004).
7. W. Sabbagh *et al.*, *Mol. Cell* **8**, 683 (2001).
8. H. D. Madhani *et al.*, *Cell* **91**, 673 (1997).
9. S. Bruckner *et al.*, *Curr. Genet.* **46**, 331 (2004).
10. S. P. Palecek *et al.*, *Genetics* **156**, 1005 (2000).
11. A. S. Nateri *et al.*, *Science* **303**, 1374 (2004).
12. M. Gao *et al.*, *Science* **306**, 271 (2004).

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Simple Foraminifera Flourish at the Ocean's Deepest Point

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Extreme water depths make it very difficult to sample the bottom of deep-ocean trenches. As a result, almost nothing is known about small sediment-dwelling organisms (meiofauna) living in these environments, which are among the most remote on Earth. During a study of western Pacific trenches (>7000 m water depth), we discovered abundant foraminifera (shelled protists) living in the Challenger Deep, the deepest place (10,896 m) in the world ocean. The fauna is dominated by morphologically simple species with organic walls. These distinctive taxa seem to be characteristic of the deepest ocean depths.

The 0- to 1-cm layer of a sediment core collected with the *KAIKO* Remote Operated Vehicle (*1*), belonging to the Japan Agency for Marine Earth Science and Technology (JAMSTEC), yielded 432 rose-bengal–stained, and therefore living, benthic foraminifera (~449 per 10 cm^2) (Fig. 1). This population density was similar to that found at our shallower (7088 to 7761 m) trench sites (table S1) and greater than at many abyssal sites (*2*). Except for four individuals of the multichambered agglutinated genera *Leptohalysis* and *Reophax*, the assemblage was dominated by delicate, soft-walled species. Most (85%) of these 428 specimens were organic-walled allogromiids, typically brown in color and with tubular morphologies, sometimes subdivided into two or more elongate or globular chambers. These morphotypes resemble the genera *Chitinosiphon*, *Nodellum*, and *Resigella*. Other taxa were spherical, organic-walled allogromiids (5.6%) and spherical and flask-like agglutinated species (psammosphaerids and saccamminids) (9.6%). Such a high proportion of organic-walled allogromiids is very unusual; in most deep-sea environments, they constitute 5 to 20% of the living assemblage (*1*). A previous study (*3*) of foraminifera from a comparable water depth reported four stained (living) individuals belonging to the agglutinated genus *Lagenammina*. Foraminifera with calcareous walls cannot exist at this great depth because the water is strongly undersaturated in calcium carbonate.

This assemblage most resembles the fauna seen at a shallower site in the Marianas Trench (7123 m water depth) (table S1). Here, *Nodellum*- and *Resigella*-like allogromiids were also common, albeit less so (45% of the assemblage) than at the Challenger Deep. Psammosphaerids, however, were more abundant (37%) and the assemblage more diverse (57 species). Samples from two northwest Pacific trenches (Kurile and Japan; 7761 m and 7088 m, respectively) yielded only a few *Nodellum*- or *Resigella*-like specimens, suggesting that the dominance of these forms in the Marianas Trench (including the Challenger Deep) may reflect a more oligotrophic setting compared with the northwest Pacific, rather than water depth. An abyssal sample (Station no. 64; 5507 m water depth) was more diverse than any of the trench material. Psammosphaerids and species of *Lagenammina* were most common, and the *Nodellum* and *Resigella* group made up about 10%, a typical proportion at abyssal depths. Studies on metazoan organisms in video records and samples from the Challenger Deep are in progress (*4*).

Analyses of small subunit ribosomal DNA gene sequences have suggested that single-chambered noncalcareous taxa form a series of deep-branching evolutionary lineages, representing the basal radiation from which more complex multichambered groups arose (*5*). They include the only foraminifera to have invaded freshwater and terrestrial (*6*) habitats. Our results indicate that these primitive groups are also important components of benthic communities in the deepest ocean trenches and may be more adaptable than other foraminiferans to extreme pressures. Similar forms are widespread in the abyssal deep sea and abundant at 7800 m water depth in the Atacama Trench (*7*), but they are never a dominant faunal component. The Challenger Deep may have developed to its present depth during the past 6 to 9 million years (*8*). Its very distinctive foraminiferal fauna probably represent the remnants of an abyssal assemblage that was able to adapt to the steady increase in hydrostatic pressure over this time period.

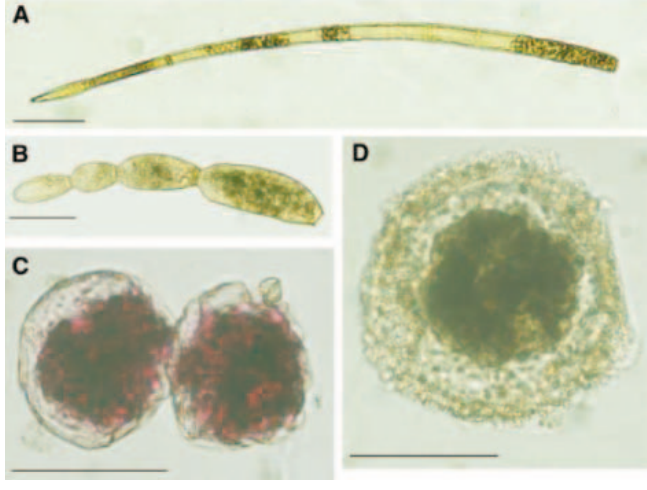

Fig. 1. Live (stained) specimens of benthic foraminifera from the Challenger Deep, part of the Izu-Bonin-Mariana Arc trench system. (**A**) A needle-shaped, organic-walled allogromiid resembling *Chitinosiphon*. (**B**) Multichambered, organic-walled allogromiids, representing a genus similar to *Resigella*. (**C**) Spherical, organic-walled allogromiids. (**D**) A spherical, agglutinated psammosphaerid. Scale bars, 50 μm.

References and Notes

1. Materials and methods are available as supporting material on *Science* Online.
2. A. J. Gooday, *J. Foraminifer. Res.* **32**, 384 (2002).
3. K. Akimoto, M. Hattori, K. Uematsu, C. Kato, *Mar. Micropaleontol.* **42**, 95 (2001).
4. J. Hashimoto, Ed., *Onboard Report KR02-13, KAIKO/KAIREI Cruise in the Challenger Deep* (JAMSTEC, Yokosuka, Japan, 2002).
5. J. Pawlowski *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 11494 (2003).
6. R. Meisterfeld, M. Holzmann, J. Pawlowski, *Protist* **152**, 185 (2001).
7. A. Sabbatini, C. Morigi, A. Negri, A. J. Gooday, *J. Micropaleontol.* **21**, 131 (2002).
8. K. Fujioka, K. Okino, T. Kanamatsu, Y. Ohara, *Geophys. Res. Lett.* **29**, 1372, 10.1029/2001 GL 013595 (2002).
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Supporting Online Material
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Materials and Methods
Table S1

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Crystal Structure of a Complex Between the Catalytic and Regulatory (RIα) Subunits of PKA

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The 2.0-angstrom structure of the cyclic adenosine monophosphate (cAMP)–dependent protein kinase (PKA) catalytic subunit bound to a deletion mutant of a regulatory subunit (RIα) defines a previously unidentified extended interface. The complex provides a molecular mechanism for inhibition of PKA and suggests how cAMP binding leads to activation. The interface defines the large lobe of the catalytic subunit as a stable scaffold where Tyr^{247} in the G helix and Trp^{196} in the phosphorylated activation loop serve as anchor points for binding RIα. These residues compete with cAMP for the phosphate binding cassette in RIα. In contrast to the catalytic subunit, RIα undergoes major conformational changes when the complex is compared with cAMP-bound RIα. The inhibitor sequence docks to the active site, whereas the linker, also disordered in free RIα, folds across the extended interface. The β barrel of cAMP binding domain A, which is the docking site for cAMP, remains largely intact in the complex, whereas the helical subdomain undergoes major reorganization.

The discovery of cAMP as a second messenger (*1*) led eventually to the identification of cAMP-dependent PKA (*2*) with regulatory subunits that are the major receptors for cAMP (*3*). PKA, ubiquitous in mammalian cells, regulates processes as diverse as growth, development, memory, metabolism, gene expression, and lipolysis. The PKA holoenzyme exists as an inactive complex of two cataytic (C) subunits and a regulatory (R) subunit dimer; binding of cAMP facilitates dissociation and activation of the C subunits.

The C subunit is comprised of a small and large lobe with the active site forming a cleft between the two lobes. This fold, first described for PKA, defines the protein kinase superfamily (*4*). The small lobe provides the binding site for adenosine 5′-triphosphate (ATP), and the large lobe provides catalytic residues and a docking surface for peptide/protein substrates. Opening and closing of the active site cleft is an essential part of catalysis (*5*). The activation loop in the large lobe contains a phosphorylation site, Thr^{197}, essential for catalysis (*6*).

Like many signaling proteins, the R subunits are modular. They contain well-ordered domains as well as disordered regions. The dimerization domain at the N terminus is a well-organized four-helix bundle that also provides a docking surface for A kinase anchoring proteins (*7*–*9*). At the C terminus of each protomer are two tandem cAMP-binding domains (CBD-A and CBD-B), each representing a structural motif that has been conserved as a binding module for cAMP from bacteria to man (*10*). Each CBD is composed of a helical subdomain and an eight-stranded β barrel where cAMP binds. The essential feature of the β barrel is a conserved phosphate binding cassette (PBC) that anchors the cAMP and shields it from solvent (*10*). The CBDs are joined to the dimerization domain by a flexible linker (*11*), which includes a substratelike inhibitor sequence that docks to the active site cleft of the C subunit in the absence of cAMP (*12*).

Whereas structures of dimerization domains and the CBDs have been solved by nuclear magnetic resonance (*7*, *9*) and crystallography (*13*, *14*), respectively, the structure of an R:C complex has been elusive. Here, we report the crystal structure of a holoenzyme complex between an oxidation-resistant form of the C subunit and a deletion mutant of RIα, RIα(91–244), which contains the inhibitor sequence and the N-terminal cAMP binding domain (CBD-A). The complex was crystallized in the presence of a nonhydrolyzable analog of ATP, adenylyl-imidodiphosphate (AMP-PNP). The structure confirms the intrasteric mechanism of regulation, defines the intricate ordering of the linker region at the interface of the RIα and C subunits, reveals a global reorganization of the helical subdomain of RIα, and shows the unliganded molecular target that is seen by cAMP. The comparison of this structure with structures of RIα in its cAMP-bound conformation provides insight into the structural basis for cAMP-induced activation of PKA.

Molecular architecture. The architecture of the RIα(91–244):C complex reveals an extended interface that covers nearly 3000 $Å^2$ (Fig. 1, A and B, and movie S1). Although the C subunit assumes a fully closed conformation with Mn_2AMP-PNP bound at the active site cleft, it does not undergo any other major conformational changes as a result of complex formation; instead, it serves as a stable scaffold for docking RIα. The docking surface on the C subunit is localized almost exclusively to the large lobe and is overlapping but distinct from the surface used for docking another PKA inhibitor, the heat stable protein kinase inhibitor (PKI) (Fig. 2D) (*15*). The binding surface extends from the inhibitor binding site at the active site cleft (site 1), across the G helix (site 2) and through to the activation loop (site 3). The electrostatic profile of this complex interface is shown in Fig. 1C.

In contrast to the C subunit, RIα undergoes major conformational changes upon complex formation (Fig. 1D and movie S2). There are three general features that describe the binding of RIα to form the holoenzyme complex. First, the inhibitor sequence docks to the active site cleft. Second, the linker segment that connects the inhibitor peptide to CBD-A is ordered. Third, CBD-A docks onto the large lobe of the C subunit (Fig. 1C). The inhibitor peptide and linker region (Arg^{94R} to Val^{112R}) are disordered in the crystal structure of cAMP-bound RIα(91–379), whereas in the complex this segment binds as an extended chain along the surface of the active site cleft (Fig. 1, A to D). In addition to this transition from disorder to order, docking of RIα results in global reorganization of the helical subdomain of CBD-A (Fig. 1D). As predicted by mutagenesis and hydrogen/deuterium exchange (*16*–*18*), this docking site involves the B helix; however, the details and magnitude of this conformational reorganization were not anticipated. The general conformation of the β barrel, which provides the binding site for cAMP in free RIα, remains largely unchanged when the C subunit binds (Fig. 1D).

Extended binding surface on the catalytic subunit. Figure 2A shows the extended surface on the C subunit that serves as a platform for docking RIα. RIα docks to at least three distinct subsites on the C subunit (Fig. 1C). Site 1 is where the basic inhibitor sequence docks to the acidic active site cleft, an interaction shared by all substrates and inhibitors of PKA. At site 2, the CBD-A, specifically the hydrophobic portion of the PBC

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containing Tyr^{205R}, binds to an extensive hydrophobic surface that surrounds Tyr^{247C} in the G helix. Finally, at site 3, the B/C helix in RIα docks to the activation loop of the C subunit. We consider site 1 first.

In this complex, AMP-PNP and RIα lock the C subunit into a fully closed conformation. The region of the linker corresponding to the inhibitor site is typically referred to as the P-3 through P+1 site (Arg^{94}-Arg-Gly-Ala-Ile^{98}), where the P site is filled by either Ser or Thr for substrates and Gly or Ala for pseudosubstrates. RIα and PKI are pseudosubstrates. As predicted from chemical modifications and from the structure of a 20-oligomer peptide containing the inhibitor consensus sequence of PKI (IP20) bound to the C subunit (*15*), this segment docks to the active site cleft in a manner similar to IP20 (Fig. 2B and fig. S3). Arg^{94R} (P-3 site) and Arg^{95R} (P-2 site) link the inhibitor sequence to several regions of the C subunit by interacting with Glu^{127C} in the linker that joins the small and large lobes, Glu^{170C} in the catalytic loop, and Glu^{230C} in the F helix (Fig. 2C and movie S3). In this closed conformation, Tyr^{330C} in the C-terminal tail interacts with the glycine-rich loop and hydrogen bonds to the 2′OH group of the ribose at the P-3 site. In addition, His^{87C} in the C helix is hydrogen bonded to the phosphate on Thr^{197C} in the activation loop. Ile^{98R} binds to the P+1 loop. This part of the inhibitor sequence in RIα forms a complementary antiparallel β strand that links the glycine-rich loop and the N terminus of the C helix to the P+1 loop to form a β sheet (Fig. 2C). The detailed interactions are summarized in Fig. 3.

In spite of sharing a common inhibitor site, IP20 and RIα use different surfaces of the C subunit to achieve high-affinity binding (Fig. 2D). Whereas the amphipathic helix of IP20 docks to a surface that lies N terminal to the inhibitor site, RIα docks to the surface that lies C terminal to the inhibitor site. As seen in Fig. 4A, binding of the P+1 residue (Ile^{98R}) to the P+1 loop not only provides a local docking site but also appears to nucleate an extended hydrophobic interface between the C subunit (site 2) and CBD-A of RIα. A key element of this surface on the C subunit is the solvent exposed Tyr^{247C} in the G helix, which is surrounded by a cluster of hydrophobic residues (Figs. 3 and 4A). Docking of Ile^{98R} to the P+1 loop specifically links this hydrophobic cluster by means of Tyr^{247C} to the hydrophobic surface surrounding Tyr^{205R} in the PBC of RIα (Figs. 3 and 4, A and B). In its cAMP-bound state, cAMP is firmly anchored to the PBC through its interactions with two key residues, Arg^{209R} and Glu^{200R} (Fig. 4C). This complex of RIα and C shows that the C subunit also competes for the PBC by the interaction of Tyr^{247C} of C with Tyr^{205R} and Ile^{98R} of RIα

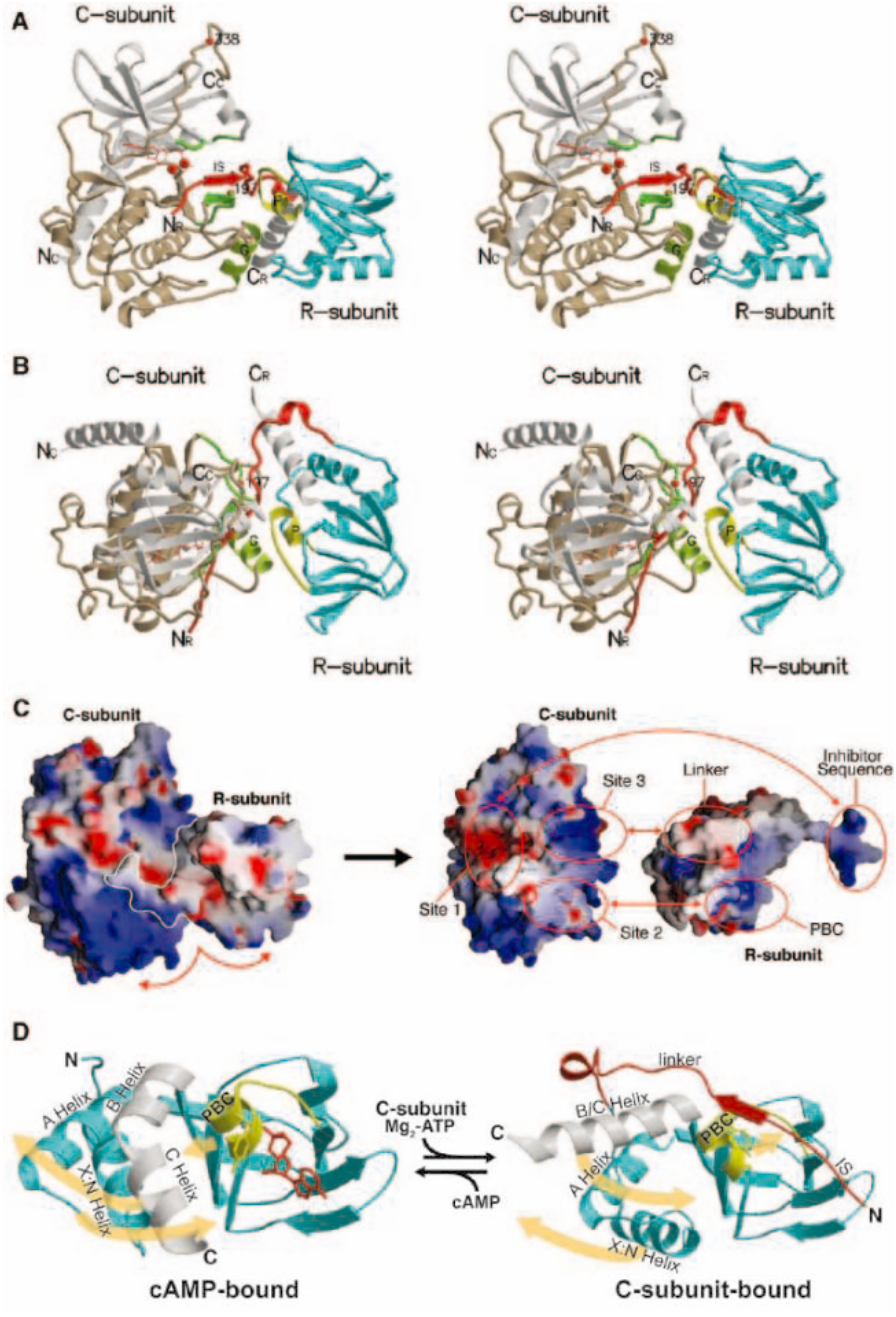

Fig. 1. Stereoview of the RIα(91–244):C:Mn_2AMP-PNP complex. (**A**) The small lobe (residues 11 to 120) and large lobe (residues 121 to 350) of the C subunit are colored in gray and tan, respectively. The N and C termini (residues 11 to 350) are labeled N_C and C_C. The activation loop, the G helix (G), and the glycine-rich loop are colored in green. Two phosphorylated residues, Thr^{197C} and Ser^{338C}, are shown as red spheres. The N and C termini (residues 91 to 242) of RIα are labeled N_R and C_R. The inhibitory/linker region (IS) (residues 91 to 112) is colored in red, the PBC (P) in yellow, the B/C helix in gray, and the rest in cyan. AMP-PNP is shown in red. This figure was generated with Molscript (*46*) and Raster3D (*47*). (**B**) Looking down the C:RIα complex, rotated 90° around a horizontal axis from the view in (A). (**C**) Electrostatic surface potential of the complex (left) and with its interface opened up to view the surfaces of individual subunits (right). The linker segment complements site 3, the PBC complements site 2, and the inhibitor site complements site 1 of the C subunit. This figure was generated with GRASS (*48*). (**D**) Structural comparison of RIα in the cAMP-bound and C-bound conformations. The inhibitory/linker region (residues 91 to 112, in red) is disordered in the cAMP-bound conformation and ordered in the C-bound conformation. The PBC (residues 199 to 210, in yellow) is stretched away from the β barrel when the C subunit binds. The B and C helices become one extended helix (residues 226 to 242, in gray) in the complex. Tyr^{205R} (yellow) at the tip of the PBC changes its orientation to interact with Tyr^{247C} at the G helix of the C subunit. This figure was generated with Molscript (*46*) and Raster3D (*47*).

(Fig. 4B). The tip of the PBC in RIα is clearly stretched by these interactions, and this stretched PBC has lost its capacity to bind cAMP (Fig. 4C). Dislodging cAMP from the PBC is probably the key rate-limiting step required for holoenzyme formation.

Site 3 on the docking surface of C is dominated by the activation loop containing Trp^{196C} and Arg^{194C}. They both interact with Glu^{105R} at the linker segment of RIα (Fig. 5A). Earlier mutagenesis (*19*) and genetic (*20*) studies indicated that Trp^{196C} would play an important role at the R:C interface. The phosphate of Thr^{197C} links the C helix by means of His^{87C} to the activation loop, and both of these segments of the C subunit interact directly with RIα. His^{87C} also interacts with Gln^{84C}, and Gln^{84C} in turn binds to Ser^{99R} in the linker region of RIα (Fig. 5A). Thus, like the glycine-rich loop, the N terminus of the C helix is an integral part of the RIα:C interface. The phosphate on Thr^{197C} is also anchored to the catalytic loop at the active site through its interactions with Arg^{165C} (Fig. 5B and fig. S2). Two regions of RIα, the C helix (Met^{234R}) and the linker (Glu^{105R}), interact with Trp^{196C} and Arg^{194C} in the activation loop (Figs. 3 and 5C).

Mutagenesis predicted that Lys^{213C} in the C subunit formed an ion pair with Glu^{143R} in RIα (*19*). The structure confirms this interaction and, in addition, shows that Lys^{213C} brings together the A and C helices, which are far apart in cAMP-bound RIα (Fig. 5D). Lys^{213C} is located in a highly conserved segment that links the activation loop with the F helix; we refer to this segment as the APE-F linker (Fig. 6A), where Glu^{208C} in the APE motif provides the conserved C-terminal anchor for the activation loop. Whereas Lys^{213C} interacts directly with RIα, two other nearby residues, Tyr^{215C} and Lys^{217C}, reach up and buttress the activation loop by hydrogen bonding to the backbone carbonyls of Thr^{197C} and Ser^{191C}, respectively. A peptide corresponding to this region was also predicted to be part of the RIα:C interface based on its protection from deuterium exchange (*17*, *18*).

Interaction sites on RIα. Based on a comparison of six cAMP-bound structures of CBDs, two elements of the CBD are predicted to be essential for signaling (*21*). The highly conserved PBC, embedded in the β-barrel subdomain, is the anchoring site for cAMP, as described earlier. The second element is the B/C helix (Fig. 6B). This element, which serves as a docking surface for interacting with other proteins or domains, is tethered to the PBC by a hydrophobic "hinge" between the B helix and the tip of the PBC (*13*, *22*). This RIα:C complex is the first time that any CBD has been seen in both a cAMP-bound state and in a cAMP-free state bound to another protein. Removing cAMP from this deletion mutant of RIα is not sufficient to induce a major conformational change (*23*). It is, rather, binding of the C subunit that induces major conformational changes (Fig. 6C).

Appreciation of RIα(91–244) in its cAMP-bound state compared with its conformation in the complex can be best achieved by focusing on three regions (Figs. 1D and 6C). We discussed many specific residues earlier, and here we consider these global changes in RIα. The charged and hydrophilic linker region, completely disordered in cAMP-bound RIα, now becomes ordered at the interface (fig. SA). The B and C helices snap into a single fully

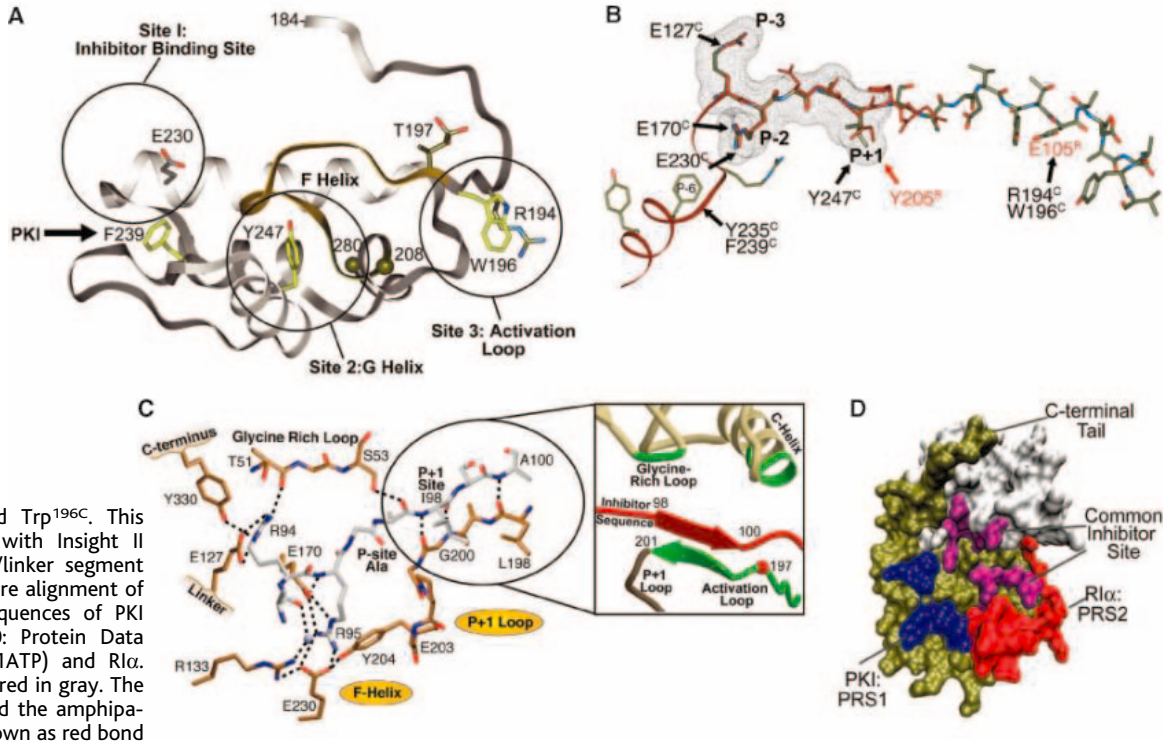

Fig. 2. Surfaces of the C subunit that interact with RIα. (**A**) The complex interface on the large lobe with the F and G helices highlighted in gray. The activation segment (residues 184 to 208) is yellow. Asp^{208C} and Arg^{280C} are each indicated by a ball. Phe^{239C} (*49*) is part of the hydrophobic docking site for PKI. Site 1 corresponds to the inhibitor docking site, site 2 to the G helix with Tyr^{247C}, and site 3 to the activation loop containing Arg^{194C} and Trp^{196C}. This figure was generated with Insight II (*50*). (**B**) The inhibitor/linker segment of RIα(91–112). Structure alignment of inhibitor consensus sequences of PKI (inhibitor peptide IP20: Protein Data Bank accession code 1ATP) and RIα. Carbons of RIα are colored in gray. The consensus sequence and the amphipathetic helix of IP are shown as red bond and ribbon, respectively. Similar to IP20 (Arg-Arg-Asn-Ala-Ile), the inhibitory sequence (Arg^{94}-Arg-Gly-Ala-Ile^{98}) of RIα blocks the active site of the C subunit. The coordinates of these residues are nearly identical with an exception of Gly/Asn substitution at the P-1 site. The linker segment interacts not only with the C subunit but with the B/C helix. Specific interacting residues from C and RIα are indicated in black and red, respectively (arrows). As in the IP20 complex, the C subunit within the complex is in the closed conformation, with a root mean square deviation of 0.42 $Å^2$ for equivalent Cα atoms. This figure was generated with Insight II (*50*). (**C**) The inhibitor region (residues 91 to 100) bound to the active site cleft (site 1). The R- and C-subunit residues are drawn with gray and tan carbons, respectively. Arg^{94R} at the P-3 site interacts with Thr^{51C} in the glycine-rich loop, Glu^{127C} in the linker, Tyr^{330C} in the C-terminal tail, and AMP-PNP. Arg^{95R} at the P-2 site is tightly held by Glu^{170C} in the catalytic loop and Glu^{230C} in the F helix (Fig. 3). Farther down toward the linker, Ile^{98R} and Ala^{100R} make antiparallel β-sheet–like interactions with the P+1 loop. This figure was generated with Visual Molecular Dynamics (VMD) (*51*) and Molscript (*46*). (**D**) Surfaces of the C subunit used for recognition of PKI and RIα. This surface rendering of the C subunit shows the small lobe in white and the large lobe (residues 128 to 350) in tan. The inhibitors of C, PKI, and the R subunits use a common surface (magenta) on which a pseudosubstrate (PKI and RI) or a substrate (RII) docks to the active site cleft. To achieve high-affinity binding requires an additional peripheral recognition site (PRS). PKI uses the surface shown in blue, designated as PBS 1, whereas RIα uses the surface shown in red (PRS2). This figure was generated with VMD (*51*).

extended helix where residues that were previously fully exposed to solvent are now embedded at the RIα:C interface. Finally, the remainder of the helical subdomain that includes the X:N helix and the A helix together with the interlinking loop rearranges to fill the space that was vacated by the movement of the C helix (Figs. 1D and 6C).

As described earlier, docking of the inhibitor site to the active site cleft results in ordering of the remaining linker. This region, highly conserved in RIα, contributes many charged residues (Figs. 1C and 2C). By interacting not only with C but also with the CBD, they function almost like a zipper to fasten the CBD-A to the large lobe of the C subunit. Most of the conformational changes in CBD-A are in the helical subdomain, with the exception of the tip of the PBC where cAMP docks. This segment is extended slightly by its interactions with Tyr^{247C} in C and Ile^{98R} in RIα (Figs. 1D, 4C, and 6C). The rest of the β barrel remains unchanged.

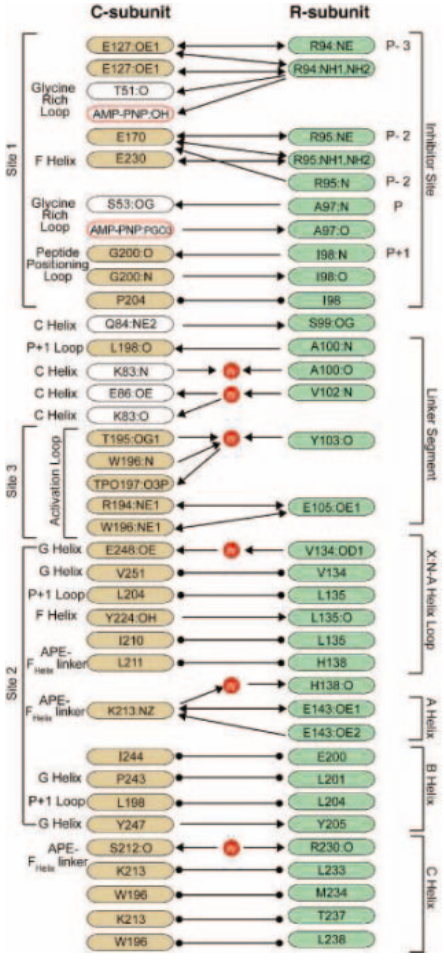

Fig. 3. Specific interactions between the R and C subunits. The location of the each residue within the complex is listed alongside of each amino acid. Solvent molecules at the interface are shown as W. Ion pair, hydrogen-bond, and van der Waals interactions are notated as ⟷, →, and ⟿, respectively.

In contrast, the helical subdomain of CBD-A undergoes major conformational changes as it docks to the surface of the C subunit. Most notably, the B and C helices become a single extended helix (Figs. 1D and 6D). Trp^{196C} in the activation loop of the C subunit helps to anchor this extended helix through its interactions with Met^{234R} in the C helix of RIα (Figs. 3 and 5C). In its cAMP-bound conformation, this entire surface of the B/C helix of the CBD-A is exposed to solvent (Fig. 1D). This surface, which includes Arg^{226R}, Arg^{230R}, Arg^{231R}, Met^{234R}, and Lys^{240R}, now becomes embedded at the interface created by the large lobe of the C subunit (Fig. 5C).

Mechanism of inhibition and activation. Understanding how a protein kinase is inhibited is as important as understanding how it functions as a catalyst. Whereas appreciation of the kinase as a catalyst has focused primarily on the dynamic small lobe and the opening and closing of the active site cleft (*5*), this structure of the RIα:C complex draws our attention to the large lobe and reveals the importance of the C subunit as a scaffold. PKA has at least seven known physiological inhibitors, four functionally nonredundant R-subunit isoforms (*24*), and three PKI isoforms (*25*); all bind to C with subnanomolar affinities. Although all share a common substrate-like inhibitor site that binds to the active site cleft, the peripheral sites that allow each to bind with high affinity are distinct (Fig. 2, B to D, and fig. S1). PKI uses a rather small surface for docking an amphipathic helix that is N terminal to the inhibitor site (*15*), whereas RIα uses a very large surface C terminal to the inhibitor site and a mechanism that involves major conformational changes in the helical region of RIα (Fig. 2D). Preliminary evidence, based on mutagenesis and hydrogen-deuterium exchange, suggests that RIIβ may use yet another variation (*26*). Collectively, these sites span a substantial surface of the large lobe and define the large lobe as a major scaffold. In spite of the extended surface, the docking sites shown in Fig. 2A are all preformed; no conformational changes are induced in the C subunit, other than the closing of the active site cleft, as a consequence of these inhibitors binding. PKI, like RIα, requires two Mg^{2+} ions and ATP to form a stable complex (*27*), and the C subunit must assume a fully closed conformation. Formation of holoenzyme with RII subunits does not require ATP.

In addition to the inhibitor site, both the G helix and activation loop of the large lobe are essential for binding to RIα. In particular,

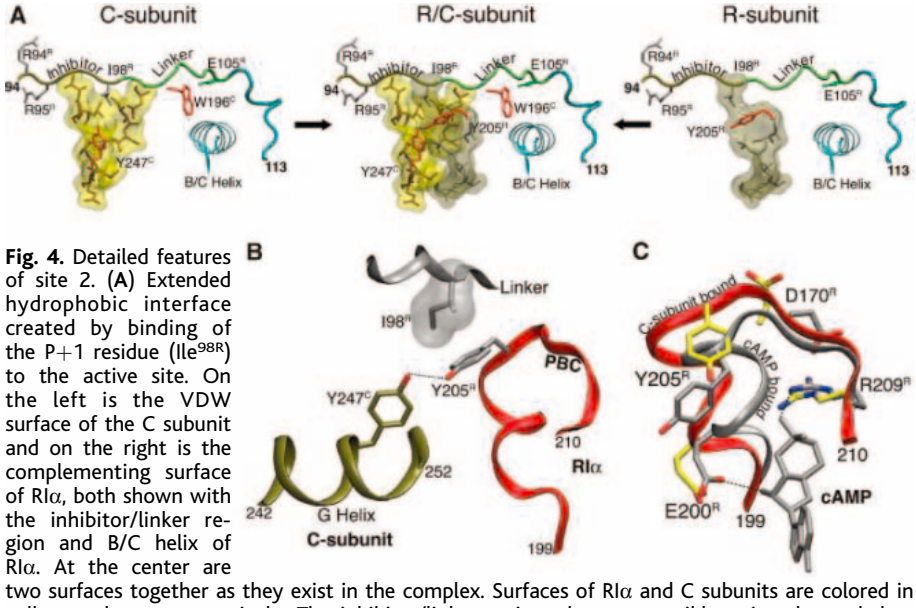

Fig. 4. Detailed features of site 2. (**A**) Extended hydrophobic interface created by binding of the P+1 residue (Ile^{98R}) to the active site. On the left is the VDW surface of the C subunit and on the right is the complementing surface of RIα, both shown with the inhibitor/linker region and B/C helix of RIα. At the center are two surfaces together as they exist in the complex. Surfaces of RIα and C subunits are colored in yellow and gray, respectively. The inhibitor/linker region, shown as a ribbon, is color coded as follows: inhibitor sequence (residues 94 to 98), tan; linker segment (residues 99 to 105), green; residues 106 to 113, cyan. In cAMP-bound RIα, this entire segment is disordered. B and C helices are also in cyan. The surface of the C subunit is composed of P+1 loop and the hydrophobic face of the G helix. Two elements of the surface within the RIα are the hydrophobic residues from the helical tip of PBC and the $X:N_{Helix}$ to A_{Helix} loop. The surface of the C subunit appears to be preformed, whereas the two elements forming the hydrophobic surface of RIα are brought together by conformational changes. (**B**) cAMP-binding site in the holoenzyme complex. Tyr^{205R} at PBC forms a hydrogen bond (dotted line) with Tyr^{247C} in the G helix. Surrounding this head-on hydrogen bonding between two tyrosines are clusters of hydrophobic residues from both subunits, as shown in (A). The side chain of the P+1 Ile nucleates this site. (**C**) The PBC (residues 199 to 210) of RIα in the cAMP-bound and C-bound conformations are aligned. The side chains, as in the C-bound conformation, are yellow, and the cAMP-bound conformations are gray. In addition to Tyr^{205R}, Asp^{170R}—which orients an important cAMP-binding residue, Arg^{209R}—changes its rotomer position when C binds. All of the images were generated with VMD (*51*).

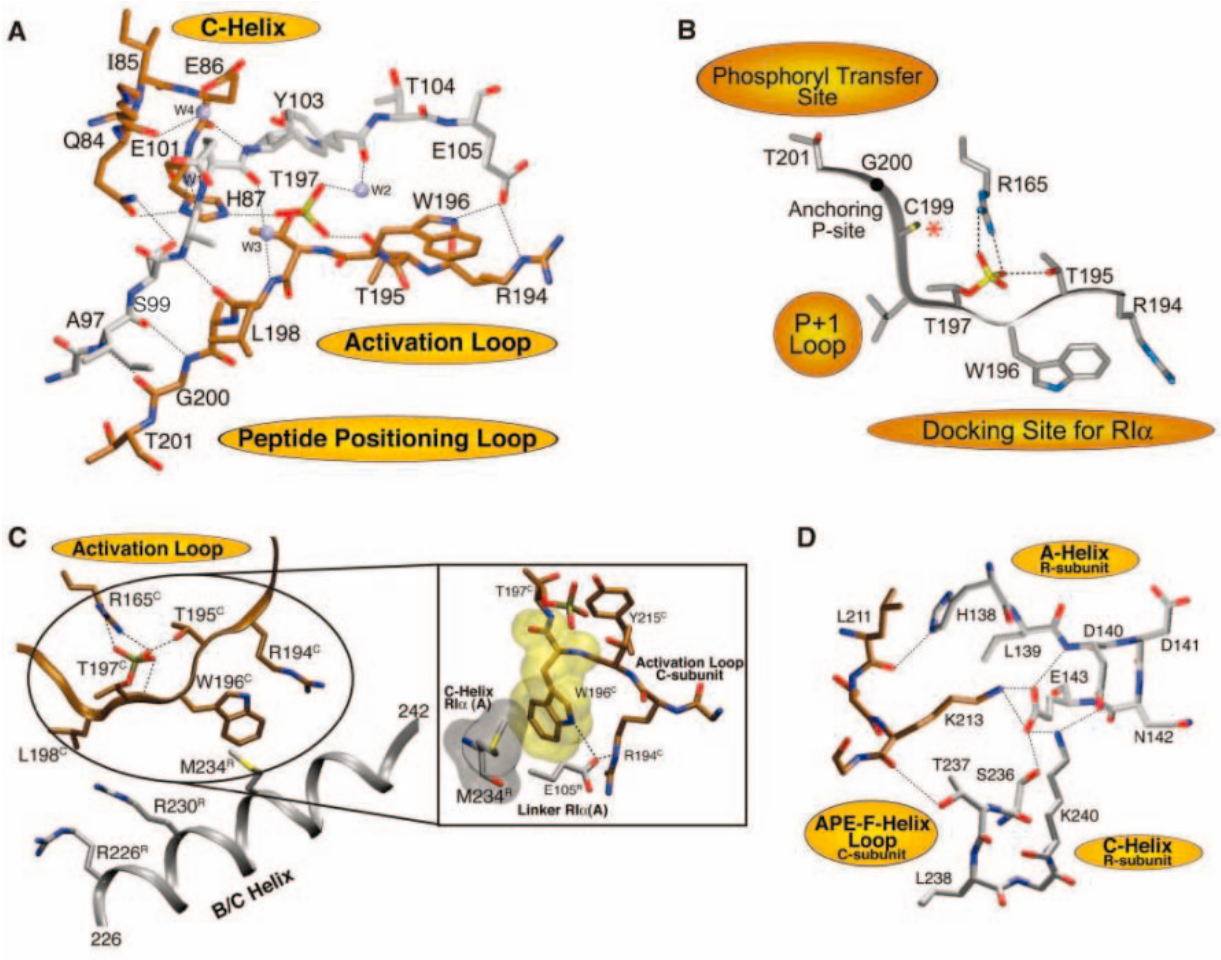

Fig. 5. Specific interactions associated with site 3. (**A**) Hydrogen-bonding network at site 3. Carbons from RIα are gray; carbons from the C subunit are tan. Specific interactions are also summarized in Fig. 3. (**B**) Interactions of the activation loop. Phosphorylation of Thr^{197C} creates parts of the RIα:C interface. Each plays a critical role. Whereas Leu^{198C}, Thr^{201C}, and Gly^{200C} contribute to recognition of the peptide, Trp^{196C} and Arg^{194C} provide a critical phosphorylation-dependent docking site for RIα. Asterisk is the reactive cystine. This figure was generated with Insight II (*50*). (**C**) Interactions between the activation loop and the B/C helix. The interface of Trp^{196C}/Arg^{194C} with the B/C helix of RIα is shown. Specific interactions between Trp^{196C}/Arg^{194C} of the C subunit and Glu^{105R}/Met^{234R} of RIα are highlighted. (**D**) Glu^{143R} in the A helix binds to Lys^{213C} of the C subunit (Fig. 3) as predicted by mutation study (*16*). The C helix and the A helix of RIα interact closely, thus helping to stabilize the inhibitory conformation. All of the images except (B) were generated with VMD (*51*).

Tyr^{247C} in the G helix and Trp^{196C} in the activation loop are important key residues that anchor RIα (Figs. 4, A and B, and 5C). Because both are highly conserved as aromatic residues in the protein kinase family, protein recognition will likely be a common feature shared by these residues in many protein kinases (*28*). The importance of the G helix as a hydrophobic docking site was demonstrated previously in the crystal structure of cdk2 bound to kinase-associated phosphatase (KAP), the phosphatase that removes the activation loop phosphate from Thr^{160} (*29*). In that complex, the phosphate on Thr^{160} of cdk2 binds directly to the catalytic site of KAP. Unlike the E, F, and H helices, which form the highly stable core of the large lobe and are extremely resistant to deuterium exchange (*30*), the G helix is more solvent accessible. Its backbone amides are only shielded from solvent when inhibitors are bound. This protection enabled us to identify this element as part of the RIα:C interface (*18*). We predict that the G helix will be a dynamic and solvent-accessible sensor for protein docking in many protein kinases.

The importance of a single phosphate in the activation loop was appreciated immediately when the first set of crystal structures was solved (*4*, *31*–*34*). In its fully phosphorylated and activated state, p-Thr^{197C} is linked to the active site through Arg^{165C} (Fig. 5B) (*35*). In inactive kinases, such as cdk2 (*31*), the loop is not phosphorylated and is typically disordered. The essential phosphorylation site on the activation loop is always coupled with an Arg-Asp (RD) motif (Arg^{165}-Asp^{166} in the C subunit), in which the equivalent to Arg^{165C} is anchored to the phosphate with Asp^{166C} positioned at the active site in the catalytic loop, where Asp^{166C} functions as a catalytic base and proton sink (*36*–*38*). Biochemical studies with mutant proteins have demonstrated the importance of the activation loop phosphorylation for catalysis (*6*, *39*, *40*). The RIα:C structure highlights another important consequence of phosphorylating the activation loop: creating a binding surface for RIα. The importance of Trp^{196C} for R-subunit inhibition was first recognized by a genetic screen designed to identify mutants that could not be regulated (*20*), and we confirmed experimentally that Trp^{196C} was an essential feature of the R:C interface (*19*). However, this is only true when the C subunit is phosphorylated on Thr^{197C}; dephosphorylated C subunit cannot bind RIα (*41*). As described earlier, Trp^{196C} is packed against residues in the B/C helix of RIα(91–244), and, similar to Tyr^{205R}, these residues are fully exposed to solvent when cAMP is bound to Δ(1–91)RIα (Figs. 5C and 6B).

The most notable revelation that emerges from the RIα:C complex is the remarkable conformational malleability of RIα. Clearly, the extended linker region needed to become ordered upon binding to the C subunit, and it

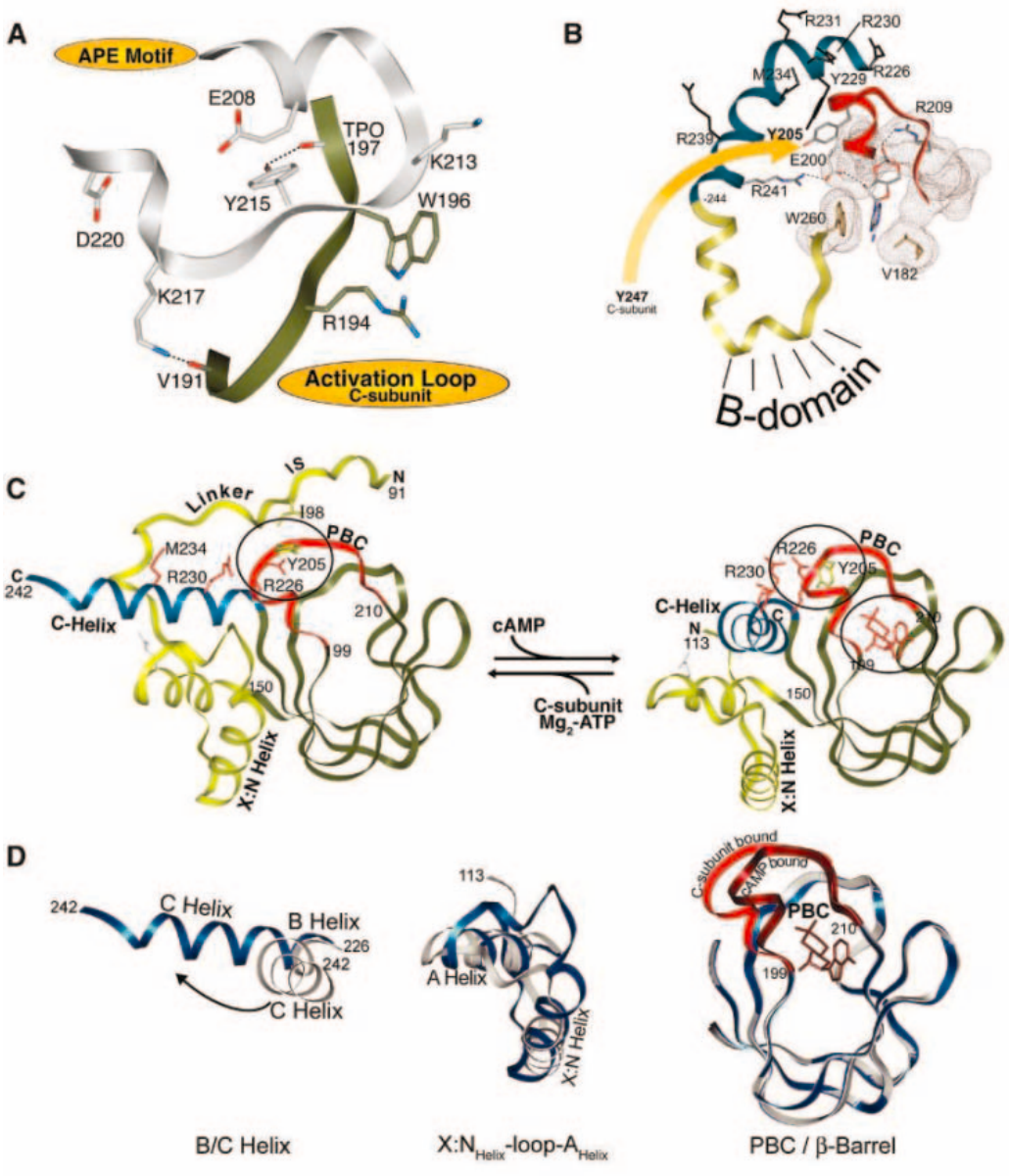

Fig. 6. (**A**) The APE-F linker motif anchors the activation loop. The activation segment and the APE-F linker motif are rendered as dark gray and light gray ribbons, respectively. (**B**) Conserved features of the CBD in RIα. CBD-A is highly conserved where two charged residues, Arg^{209R} and Glu^{200R}, anchor the cAMP. Hydrophobic residues, shown with the van der Waals surfaces, surround the cAMP and shield the adenine ring. The B and C helices (cyan) interface with the PBC (red) through a hydrophobic hinge at the B helix and through hydrophobic capping of the adenine ring by Trp^{260R}. In our construct, residues 248 to 260 (gold) are missing. This figure was adapted from figure 7C of (*21*). (**C**) Comparison of RIα in its cAMP-bound (residues 113 to 242) (*14*) and C subunit–bound (residues 91 to 242) conformations. The C subunit–bound conformation is on the left and cAMP-bound conformation is on right. The inhibitory/linker region and X:N and A helices are in yellow. PBC is in red and the B and C helices are in cyan. (**D**) Structure alignment of the two conformations of RIα. Specific conformational changes associated with the B and C helices (residues 226 to 242), the X:N and A helices (residues 113 to 150), and the β barrel (residues 151 to 225) are shown. The C-bound conformation is in cyan and the cAMP-bound conformation is in gray with PBC colored red in both conformations. The β-barrel subdomains minus the PBC (residues 151 to 200 and 210 to 225) of the two conformations align with a Cα root mean square deviation of 1.05 $Å^2$, whereas the helical subdomains do not align. All of the figures were generated with Insight II (*50*).

was not possible to predict the molecular features of this docking in advance of the structure. However, the reorganization of the helical subdomain was not anticipated and the magnitude of the conformational changes is substantial (Figs. 1D and 6C). Although the β barrel that provides the docking site for the phosphate of cAMP remains largely intact, the helical subdomain is completely reorganized. Understanding the dynamics and the pathway for this change is another challenge for the future. We predict that in the absence of cAMP, the helical subdomain should be quite dynamic. The molecule is much less stable, and unfolding is no longer cooperative when cAMP is removed (*42*, *43*). A stable conformation is created only in the presence of cAMP or C subunit. This mechanism of stabilizing very different conformations by either a small molecule ligand or another protein has not been observed previously.

Ligand-induced activation of the RIα holoenzyme is a highly cooperative and ordered allosteric process with cAMP binding first to CBD-B and then to CBD-A (*44*). Binding of cAMP to CBD-A then leads to release of the C subunit. In the complex examined here, CBD-B is missing. This domain is tightly anchored by multiple hydrophobic interactions to the segment that follows the C helix (residues 245 to 259). Trp^{260R} at the beginning of the A helix of CBD-B provides the hydrophobic cap for the adenine ring of cAMP bound to CBD-A (Fig. 6B). Thus, when cAMP is released from the CBD-A, it removes a major hydrophobic anchor between the two domains, as predicted by Berman *et al.* (*21*). Because CBD-B is tightly anchored to residues 245 to 259 (*14*), it will move along with the C helix of CBD-A when the C subunit is bound. The conformational changes in the helical subdomain of CBD-A in RIα, as revealed in this complex, thus predict that there will be a major change in the orientation of CBD-B relative to CBD-A when RIα is bound to the C subunit. A notable conformational change was observed from the neutron scattering studies of the RIα holoenzyme compared to the RIα homodimer, and this data can now be modeled more precisely with the availability of this RIα:C complex (*45*). Another challenge now will be to confirm the spatial and temporal features of these global changes in conformation in the larger protein.

References and Notes

1. E. W. Sutherland, T. W. Rall, *J. Biol. Chem.* **232**, 1077 (1958).
2. D. A. Walsh, J. P. Perkins, E. G. Krebs, *J. Biol. Chem.* **243**, 3763 (1968).
3. G. N. Gill, L. D. Garren, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 786 (1971).
4. D. R. Knighton *et al.*, *Science* **253**, 407 (1991).
5. D. A. Johnson, P. Akamine, E. Radzio-Andzelm, Madhusudan, S. S. Taylor, *Chem. Rev.* **101**, 2243 (2001).

6. J. A. Adams, M. L. McGlone, R. M. Gibson, S. S. Taylor, *Biochemistry* **34**, 2447 (1995).
7. P. Banky *et al.*, *J. Mol. Biol.* **330**, 1117 (2003).
8. M. G. Newlon *et al.*, *EMBO J.* **20**, 1651 (2001).
9. M. G. Newlon *et al.*, *Nature Struct. Biol.* **6**, 222 (1999).
10. J. M. Canaves, S. S. Taylor, *J. Mol. Evol.* **54**, 17 (2002).
11. J. A. Hauer, P. Barthe, S. S. Taylor, J. Parello, A. Padille, *Protein Sci.* **8**, 545 (1999).
12. J. D. Corbin *et al.*, *J. Biol. Chem.* **253**, 3997 (1978).
13. T. C. Diller, N. H. Xuong, S. S. Taylor, *Structure* **9**, 73 (2001).
14. Y. Su *et al.*, *Science* **269**, 807 (1995).
15. D. R. Knighton *et al.*, *Science* **253**, 414 (1991).
16. R. M. Gibson, Y. Ji-Buechler, S. S. Taylor, *J. Biol. Chem.* **272**, 16343 (1997).
17. G. S. Anand, C. A. Hughes, J. M. Jones, S. S. Taylor, E. A. Komives, *J. Mol. Biol.* **323**, 377 (2002).
18. G. S. Anand *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13264 (2003).
19. R. M. Gibson, S. S. Taylor, *J. Biol. Chem.* **272**, 31998 (1997).
20. S. A. Orellana, G. S. McKnight, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4726 (1992).
21. H. M. Berman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 45 (2005).
22. H. Rehmann *et al.*, *Nature Struct. Biol.* **10**, 26 (2003).
23. C. Kim, unpublished data.
24. E. P. Brandon, R. L. Idzerda, G. S. McKnight, *Curr. Opin. Neurobiol.* **7**, 397 (1997).
25. D. A. Walsh, K. L. Angelos, S. M. Van Patten, D. B. Glass, L. P. Garetto, in *Peptides and Protein Phosphorylation*, B. E. Kemp, Ed. (CRC Press, Boca Raton, FL, 1990), pp. 43–84.
26. D. Law, G. Anand, E. A. Komives, S. S. Taylor, L. F. Ten Eyck, in preparation.
27. F. W. Herberg, M. L. Doyle, S. Cox, S. S. Taylor, *Biochemistry* **38**, 6352 (1999).
28. S. K. Hanks, T. Hunter, *FASEB J.* **8**, 576 (1995).
29. H. Song *et al.*, *Mol. Cell* **7**, 615 (2001).
30. S. M. G. Jie Yang, Mike S. Deal, Ganesh S. Anand, Virgil L. Woods, S. S. Taylor, *J. Mol. Biol.* (2004).
31. H. L. De Bondt *et al.*, *Nature* **363**, 595 (1993).
32. S. S. Taylor, E. Radzio-Andzelm, *Structure* **2**, 345 (1994).
33. F. Zhang, A. Strand, D. Robbins, M. H. Cobb, E. J. Goldsmith, *Nature* **367**, 704 (1994).
34. D. J. Owen, M. E. Noble, E. F. Garman, A. C. Papageorgiou, L. N. Johnson, *Structure* **3**, 467 (1995).
35. C. Venien-Bryan *et al.*, *Structure* **10**, 33 (2002).
36. F. Li *et al.*, *J. Mol. Biol.* **315**, 459 (2002).
37. B. Nolen, S. S. Taylor, G. Ghosh, *Mol. Cell* **15**, 661 (2004).
38. M. Valiev, R. Kawai, J. A. Adams, J. H. Weare, *J. Am. Chem. Soc.* **125**, 9926 (2003).
39. J. A. Adams, *Chem. Rev.* **101**, 2271 (2001).
40. L. M. Stevenson, M. S. Deal, J. C. Hagopian, J. Lew, *Biochemistry* **41**, 8528 (2002).
41. G. H. Iyer, M. J. Moore, S. S. Taylor, *J. Biol. Chem.*, in press.
42. J. M. Canaves, D. A. León, S. S. Taylor, *Biochemistry* **39**, 15022 (2000).
43. D. A. León, W. R. G. Dostmann, S. S. Taylor, *Biochemistry* **30**, 3035 (1991).
44. D. Øgreid, S. O. Døskeland, *FEBS Lett.* **129**, 287 (1981).
45. W. T. Heller *et al.*, *J. Biol. Chem.* **279**, 19084 (2004).
46. P. J. Kraulis, *J. Appl. Crystallogr.* **24**, 946 (1991).
47. E. A. Merritt, D. J. Bacon, *Methods Enzymol.* **277**, 505 (1997).
48. M. Nayal, B. C. Hitz, B. Honig, *Protein Sci.* **8**, 676 (1999).
49. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
50. Insight II, Accelrys, San Diego, CA (2003).
51. W. Humphrey, A. Dalke, K. Schulten, *J. Mol. Graph.* **14**, 33 (1996).

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Supporting Online Material

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Saturn's Temperature Field from High-Resolution Middle-Infrared Imaging

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Saturn was imaged between 8 and 24.5 micrometers at ~3000-kilometer resolution with the Keck I Telescope. Saturn's atmosphere has zonal temperature bands, which are mostly uncorrelated with visible cloud reflectivity, strong 100-millibar zonal temperature oscillations near 32°S, a warm south polar cap, and a compact hot point within 3° of the south pole.

Saturn's atmosphere in the thermal infrared has hemispherically asymmetric thermal properties (*1*), which indicate the strong influence of seasonal radiative forcing. It also has heterogeneous and time-variable cloud layers sensed at 5.2 μm (*2*). These demonstrate the role of dynamics in creating inhomogeneities in a cloud system (which is deeper than the haze layer, which makes its visual appearance so featureless). By imaging at longer wavelengths that are dominated by the opacity of the well-mixed gases CH_4 and H_2, we mapped Saturn's stratospheric and upper tropospheric temperature fields in order to quantify and understand the influences of dynamics and radiation on the atmosphere and their similarities with and differences from the atmosphere of Jupiter. We expected substantial differences in seasonal radiative forcing, given that Saturn's obliquity is 24° as compared with Jupiter's 3°. But we also wanted to document manifestations of dynamical forces which might be similar to or different from Jupiter, such as the degree of correlation between Saturn's thermal and cloud fields, the presence of longitudinal temperature variations (including waves), and the appearance of polar vortex phenomena.

We imaged Saturn at middle-infrared wavelengths during 6 cumulative hours on the night of 3 February 2004, at the Keck I Telescope, using the Long Wavelength Spectrometer (LWS), a facility middle-infrared camera-spectrometer in its imaging mode. Because angular resolution in the middle infrared is limited by diffraction rather than terrestrial atmospheric turbulence, a large primary mirror provides unprecedented spatial resolution. Our characteristic 0.5–arc sec angular resolution projected to 3000 km at Saturn's distance of about 10 astronomical units. Using the LWS 10-by-10–arc sec field of view, we created mosaics of Saturn using narrow-band filters with effective wavelengths of 8.00, 17.65, 18.75, 23.10, and 24.50 μm. Only at 17.65 μm was the planet successfully mosaicked a second time, extending our coverage to 270° of longitude.

The filters chosen are sensitive to CH_4 emission (8.00 μm) from the 3-mbar region of Saturn's stratosphere and to H_2 emission (17.65 to 24.50 μm) from the 80- to 200-mbar region of Saturn's upper troposphere and lower stratosphere, near the tropopause (fig. S1). We used the five filtered observations to derive temperatures at two levels, near atmospheric pressures of 3 mbar and 100 mbar, because the contribution functions for the upwelling radiance for the 17.65- to 24.50-μm filters, clustered around atmospheric levels of 80 to 200 mbar of pressure, were highly overlapping and widely separated from the 8.00-μm filter contribution function peak near 3 mbar (fig. S2). We derived temperatures at each of these levels, using a model perturbation approach similar to those used to derive temperatures on Jupiter (*3*, *4*). Initial model temperature profiles were based on Voyager Infrared Interferometer Spectrometer (IRIS) (*5*–*8*) and Composite Infrared Spectrometer (CIRS) (*9*) results. To simplify language in this report, we refer to the 3-mbar and the 100-mbar levels as the stratosphere and troposphere, respectively (*10*).

The primary distinctive feature of the stratospheric temperature field (Figs. 1 and 2)

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is its hemispherical asymmetry, with temperatures increasing monotonically toward the south pole. This is expected as a result of seasonal forcing, because Saturn is just past the southern summer solstice. Some time-dependent radiative-convective model results (*11*, *12*), shown for this season in Fig. 2, are consistent with the general trend of warming toward higher southern latitudes, but fail to predict the distinctive warming between 70°S (planetocentric) and the pole.

A steep temperature increase of ~2 K takes place at 100 mbar between 69° and 74°S planetocentric latitude, correlated with an increase of ~5 K at 3 mbar (Fig. 2). At 100 mbar, another marked rise of some ~2.5 K takes place between ~87° and 90°S, with a correlated increase of ~1 K at 3 mbar. These regions of elevated temperatures are correlated with lower visible reflectivities. Thus, the visibly dark polar spot is coincident with the highest temperatures measured in the atmosphere. The properties of the broad polar cap are suggestive of polar vortices on other planets (*13*–*16*), but instead of anomalously cold temperatures, the southern polar temperatures on Saturn are warm, sustained by nearly 15 years of continuous illumination. The existence of a zonal jet at planetocentric latitude 69°S (*17*) is consistent with this interpretation. This explanation would predict a cold vortex at Saturn's north pole, having undergone radiative cooling for nearly 15 years, a phenomenon that can be verified by Cassini's thermal spectrometer CIRS in the near future.

The appearance of a compact region of maximum temperatures within 3° latitude of the south pole is unexpected, having no long-term analog in other planetary atmospheres (*18*). The enhanced temperatures could be explained by local radiative heating by stratospheric particulates entrained in near-polar and polar regions, consistent with the increase of stratospheric particulates derived from analysis of cloud reflectivity (*17*), but we must still invoke dynamics to keep the stratospheric particles themselves entrained in such compact areas. Thus, dynamical as well as radiative forcing is required to explain the strong temperature enhancement. In fact, heating of the polar atmosphere by forced downwelling of relatively dry air is also consistent with the deeper tropospheric clouds sensed at the pole (*17*).

The organization of stratospheric and tropospheric temperatures over lower latitudes is dominated by zonal bands of alternating warm and cool temperatures, with temperature differences higher in the troposphere than the stratosphere (Fig. 2). The area of lowest tropospheric temperatures near the equator coincides with a region of higher cloud reflectivity and wind speed. The cold region has been noted in earlier studies of Saturn's temperature from Pioneer 11 (*19*) and Voyager 1 (*20*).

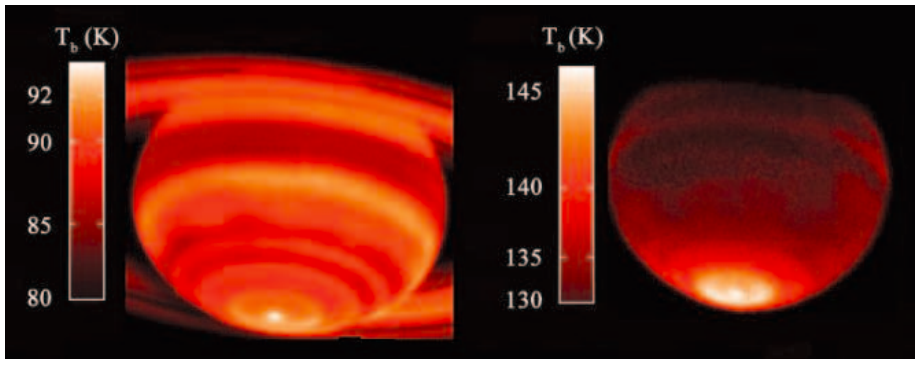

Fig. 1. Examples of Saturn mosaics at 17.65 μm (**left**) and 8.00 μm (**right**), with the appropriate brightness temperatures (T_b) given by the scale bar. Ring obscuration affects the northern hemisphere in both images, but they are too faint to be seen against the sky at 8.00 μm.

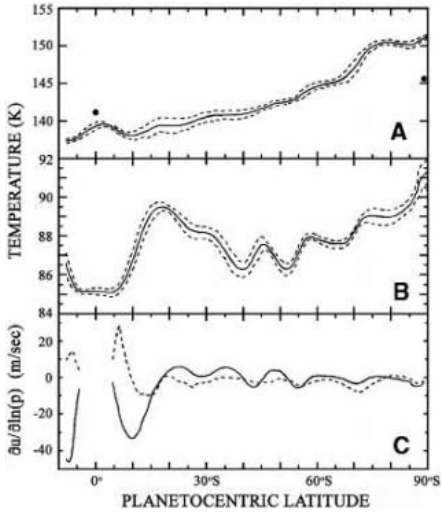

Fig. 2. Meridional variation of zonally averaged properties of Saturn's atmosphere. The top two panels show temperatures at 3 mbar (**A**) and 100 mbar (**B**). The extent of ±2 SD limits is shown in the 3-mbar plot, and the extent of ±1 SD limits is shown in the 100-mbar plot. The two solid circles in (A) represent temperatures predicted by time-dependent radiative-convective models (*11*, *12*) at the equator and south pole for the date of these observations. (**C**) The vertical gradient of the zonal wind velocity, *u*, per atmospheric scale height, $\partial u/\partial \ln(p)$, at two pressure levels, derived from the meridional gradient of temperature, assuming geostrophic balance.

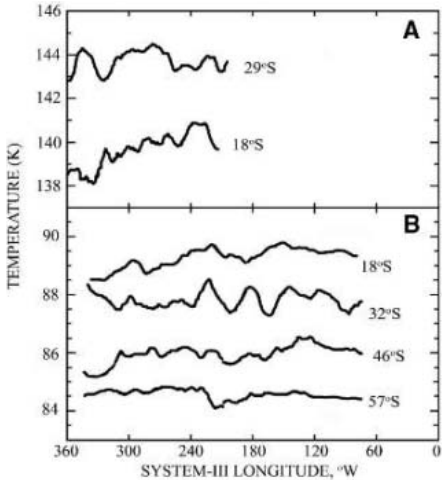

Fig. 3. Zonal temperature variations in the stratosphere (**A**) and troposphere (**B**). The temperatures at 46° and 57°S are offset to lower temperatures at 1.5° and 3.5° latitude, respectively. Tropospheric temperatures cover nearly double the longitude range of the stratospheric temperatures as a result of the second mosaic taken at 17.65 μm.

Between 20° and 50°S, there are no correlations between tropospheric and stratospheric temperatures or between temperatures and visible reflectivity.

On the other hand, the instantaneous geostrophic wind shear at 100 mbar, computed from the thermal wind approximation for nonzero latitudes, is correlated with prograde (positive, eastward) zonal cloud-tracked wind speeds (*21*–*23*) between 20° and 70°S. Near 20°S, both the 3-mbar and 100-mbar wind shears are negative, consistent with a Jupiter-like falloff of winds with altitude (*24*). However from 5° to 7°S, the 3-mbar wind shear has the opposite sign of the 100-mbar wind shear; thus, the prograde wind speed gradient increases with altitude, although not sufficiently to change the sign of the wind speed at 3 mbar. Between 20° and 50°S, the stratospheric wind shear is closer to zero than in the troposphere, consistent with a diminishing wind with altitude.

Most of the zonal bands are axisymmetric, but zonal (longitudinal) variability is evident in the images (Fig. 1), and its presence can be detected by the standard deviation associated with zonal mean temperatures (Fig. 2). The highest amplitude zonal oscillations of the tropospheric temperature field are near 32°S (Fig. 3). We analyzed the power spectrum of these oscillations using a Lomb-Scargle periodogram, similar to earlier studies of Jupiter (*3*, *4*) and Saturn (*20*) which were characterized by irregular or incomplete longitudinal sampling. The power spectrum of this structure is dominated by wavenumbers 9 to

10. Similar zonal temperature variability is seen as far as 4° north and south of this latitude at lower amplitude (Fig. 1). Zonal oscillations of lower amplitude are also present in the troposphere and stratosphere at other latitudes (Fig. 3). The power spectra of these regions show no components rising above the 99% false-alarm probability level.

Zonal temperature oscillations on Saturn were detected in Voyager 1 infrared maps (*20*), and a similar phenomenon has been seen on Jupiter (*3*, *4*, *25*). The oscillations on Jupiter were shown to be thermal waves that are moving slowly with respect to the rotation of the interior of the planet and are uncorrelated with the zonal winds associated with Jupiter's cloud system (*25*); a similar conclusion was reached regarding Saturn (*20*). Similar ground-based and Cassini CIRS observations will be needed to determine the phase speed of the thermal waves.

References and Notes

1. F. C. Gillett, G. S. Orton, *Astrophys. J.* **195**, L47 (1975).
2. P. A. Yanamandra-Fisher, G. S. Orton, B. M. Fisher, A. Sánchez-Lavega, *Icarus* **150**, 189 (2001).
3. G. S. Orton *et al.*, *Science* **252**, 537 (1991).
4. G. S. Orton *et al.*, *Science* **265**, 625 (1994).
5. R. A. Hanel *et al.*, *Science* **212**, 192 (1981).
6. R. A. Hanel *et al.*, *Science* **215**, 544 (1982).
7. J. Pirraglia, B. J. Conrath, M. D. Allison, P. J. Gierasch, *Nature* **292**, 677 (1981).
8. B. J. Conrath, P. J. Gierasch, E. Ustinov, *Icarus* **135**, 501 (1998).
9. F. M. Flasar *et al.*, *Science*, published online 23 December 2004 (10.1126/science.1105806).
10. Details of the temperature retrieval process are described in the supporting material on *Science* Online.
11. B. Bézard, D. Gautier, B. Conrath, *Icarus* **50**, 274 (1984).
12. B. Bézard, D. Gautier, *Icarus* **61**, 296 (1985).
13. See (*14*) for Earth's Antarctic vortex.
14. J. R. Schoeberl, D. R. Hartmann, *Science* **251**, 46 (1991).
15. See (*16*) for Titan's polar vortex.
16. F. M. Flasar, *Planet. Space Sci* **46**, 1125 (1998).
17. A. Sánchez-Lavega, S. Pérez-Hoyos, J. R. Acarreta, R. G. French, *Icarus* **160**, 216 (2002).
18. The closest analog is the sudden polar warming phenomenon in Earth's atmosphere (see, for example, R. J. Reed, J. L. Wolfe, and H. Nishimoto. *J. Atmos. Sci.* **20**, 2556, 1963), but it is a very short-term phenomenon, unlike Saturn's warm polar region, as suggested by several years of lower-resolution thermal imaging from the 3-m NASA Infrared Telescope Facility.
19. A. P. Ingersoll, G. S. Orton, G. Munch, G. Neugebauer, S. C. Chase, *Science* **207**, 434 (1980).
20. J. R. Achterberg, F. M. Flasar, *Icarus* **119**, 350 (1996).
21. A. Sánchez-Lavega, R. Hueso, S. Pérez-Hoyos, J. F. Rojas, R. G. French, *Icarus* **170**, 519 (2004).
22. A. Sánchez-Lavega, J. F. Rojas, P. V. Sada, *Icarus* **147**, 405 (2000).
23. A. Sánchez-Lavega, R. Hueso, S. Pérez-Hoyos, J. F. Rojas, R. G. French, *Nature* **423**, 623 (2003).
24. P. J. Gierasch, J. A. Magalhães, B. J. Conrath, *Icarus* **67**, 456 (1986).
25. J. A. Magalhães, A. L. Weir, P. J. Gierasch, B. J. Conrath, S. S. Leroy, *Icarus* **88**, 39 (1990) and references therein.

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Supporting Online Material

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SOM Text
Fig. S1 and S2
References

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REPORTS

Rapid Formation of Sulfuric Acid Particles at Near-Atmospheric Conditions

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We investigated the formation of new particles in a laboratory study, starting from H_2SO_4 produced in situ through the reaction of OH radicals with SO_2. Newly formed particles were observed for H_2SO_4 concentrations above 7×10^6 per cubic centimeter. At 293 kelvin, a rough estimate yielded a nucleation rate of 0.3 to 0.4 particles per cubic centimeter per second for $\sim 10^7$ particles per cubic centimeter of H_2SO_4 (particle size $\geq$ 3 nanometers). These findings are in agreement with observations from the atmosphere. The results demonstrate that under laboratory conditions similar to the atmosphere, particle formation occurs at atmospheric H_2SO_4 concentration levels.

Mass balances of the atmospheric aerosol have been used for more than 30 years (*1*) to identify potential human influence on climate. This is appropriate for mass-dominated sources of primary aerosol particles, such as Earth's crust or the sea surface, and their influence on processes controlled by particulate mass, such as iron fertilization of southern oceans (*2*). On the other hand, crucial processes such as cloud formation (*3*) and possibly even certain health effects (*4*) are controlled by the number of aerosol particles, including secondary particles. For several reasons, the source processes controlling the number of secondary particles in the atmosphere are much more complex and difficult to understand than those of the primary particles: (i) a host of gas-phase particle precursors with their respective formation processes is involved; (ii) particle nucleation from the gas phase may possibly comprise several species concurrently, including ions; and (iii) there are no experimental techniques available to size, quantify, or chemically specify the newly formed particles ab initio.

Sulfuric acid is a gas-phase particle precursor that has been implicated for many years as a major atmospheric nucleating species (*5*). Laboratory data on binary sulfuric acid nucleation rates with water, however, are insufficient by many orders of magnitude in explaining atmospheric nucleation (*6*–*8*). Ternary nucleation, involving the additional ubiquitous species ammonia, has been suggested from theoretical considerations as an explanation for observed particle nucleation in the atmosphere (*9*, *10*). Further possible routes for atmospheric particle formation are nucleation processes involving iodine oxide for coastal areas (*11*, *12*), ion-induced nucleation (*13*), and nucleation assisted by products from the atmospheric oxidation of aromatics (*14*).

We report results from laboratory experiments conducted in an atmospheric pressure flow-tube that was irradiated with ultraviolet (UV) lamps when required (*15*). In our first experiments, we took H_2SO_4 from a liquid reservoir by passing a part of the dry carrier gas through a H_2SO_4 saturator (97.5%, Fluka), and mixed this gas stream with humidified carrier gas at the entrance of the flow tube.

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Particle formation took place for H_2SO_4 concentrations above 10^{10} cm^{-3}, which is in line with observations from other researchers using H_2SO_4 directly from a liquid reservoir (*6*–*8*).

Further experiments applied in situ gas-phase formation of H_2SO_4 in the flow tube (Fig. 1). The chosen pathways leading to H_2SO_4 are equal to those in the atmosphere (*16*). O_3 was photolyzed, forming $O(^1D)$ atoms, and the subsequent reaction with water vapor produced OH radicals. The OH radicals attacked SO_2, and after the reaction with O_2, the adduct $HOSO_2$ yielded SO_3 and HO_2. In the reaction of SO_3 with water vapor, two molecules of H_2O or one dimer of H_2O per molecule of SO_3 are needed (*17*, *18*), which finally led to H_2SO_4. A hydrocarbon (at first furan) was added for OH radical titration. Formation of new particles was visible only in the presence of all reactants needed for H_2SO_4 formation, including UV radiation for OH radical production. The lack of particles in the absence of SO_2 indicates that the reaction of OH radicals with furan does not produce new particles itself. This fact was confirmed for all hydrocarbons applied. The lack of particles in the absence of O_3 indicates that no further photolytic processes are forming particles.

To determine the H_2SO_4 concentration in the flow tube, we used a simple kinetic model.

$$\mathrm{OH} + \mathrm{HC\ (furan)} \rightarrow \mathrm{products} \quad (1)$$

$$\mathrm{OH} + \mathrm{SO_2} \rightarrow \ldots. \rightarrow \mathrm{H_2SO_4} \quad (2)$$

$$\mathrm{H_2SO_4} \rightarrow \mathit{wall} \quad (3)$$

Initial concentrations of the hydrocarbons (HC; *cyclo*-hexane, *n*-heptane, mesitylene, and furan) and SO_2 were chosen so that more than 99% of the OH radicals reacted via pathway 1. That allowed the determination of the OH radical concentration in the irradiated zone of the flow tube from the measured amount of consumed HC (*19*). With this knowledge, H_2SO_4 concentrations could be easily calculated by considering pathways 2 and 3. The corresponding rate coefficients were taken from literature (*20*–*23*).

In Fig. 2, experimentally observed particle numbers for calculated H_2SO_4 concentrations are plotted with different hydrocarbons (*cyclo*-hexane, *n*-heptane, mesitylene, and furan) for OH radical titration [relative humidity (r.h.) = 28%]. The stated H_2SO_4 concentration is calculated for the end of the irradiated zone. A concentration profile inside the flow tube is given in fig. S1. In contrast to the experiments using H_2SO_4 from a liquid reservoir, in which particle formation started at 10^{10} cm^{-3} of H_2SO_4, here formation of new particles had already begun at H_2SO_4 concentrations of 7×10^6 cm^{-3}. According to a rough estimate, the uncertainty of these calculated H_2SO_4 concentrations is about a factor of 2. The slopes of the particle number versus H_2SO_4 concentration curves (here between 3 and 5 depending on r.h.) give the number of the H_2SO_4 molecules in the critical cluster according to the nucleation theorem (*24*). However, this number might be overestimated. This is because (i) particle diameters measured at the end of the flow tube are ~3 nm and increase with increasing H_2SO_4 concentration; and (ii) for particle diameters ~3 nm, the particle detection efficiency increases with increasing particle size. The combination of these two facts yields increasing detection efficiencies with increasing H_2SO_4 concentrations, i.e., artificially larger slopes in Figs. 2 and 3.

The data points obtained for the different hydrocarbons show very good agreement, indicating that the different reaction products from pathway 1 do not participate in the nucleation process substantially or influence the nucleation process in a nearly analogous manner. Reaction products arising from *cyclo*-hexane are mainly *cyclo*-hexanone and *cyclo*-hexanol (*25*), with final concentrations on the order of magnitude of 10^{11} cm^{-3} or less. For *n*-heptane, mesitylene, and furan, information in the literature concerning product formation is sparse.

The finding that particle formation takes place for H_2SO_4 concentrations of ~10^7 cm^{-3} is supported by experiments using ozonolysis of olefins as a "dark" OH radical source (*26*). Formation of new particles was detected for ~2×10^7 cm^{-3} of H_2SO_4 at r.h. = 28%, nearly independently of the olefin used. In these experiments, the reaction pattern describing H_2SO_4 formation was more complex and not all kinetic coefficients were available, making rough estimates necessary. Considering the possible uncertainties in these experiments, e.g., a factor of 2 to 4 with respect to the calculated H_2SO_4, the agreement of the results from ozonolysis (*26*) with those from photolysis is good.

For a fixed H_2SO_4 concentration, the number of new particles increased for increasing r.h. (Fig. 3). The increase was much more pronounced for the lowest r.h. values. At r.h. = 11%, ~4×10^7 cm^{-3} of H_2SO_4 were needed to produce detectable numbers of particles. For higher r.h. values (28% and 49.5%), the differences in the number of formed particles were small, close to the uncertainty of the measurement. Previous studies with H_2SO_4 from a liquid reservoir revealed similar r.h. dependencies, especially for low r.h. values (*6*, *8*).

Atmospheric data from simultaneous measurements of newly formed particles and H_2SO_4 at ground level are rather rare. However, existing data sets show consistently that ~10^7 cm^{-3} of H_2SO_4 are necessary in the atmosphere for particle formation (*27*–*29*). This is exactly the order of magnitude of

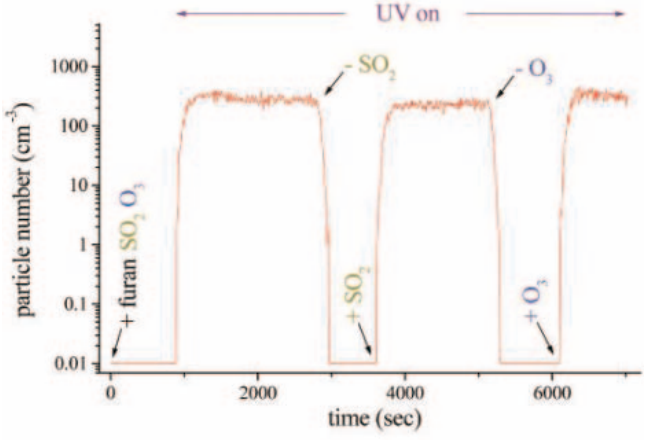

Fig. 1. Measured particles (particle size ≥ 3 nm) from a typical experiment for different trace gas additions, at 293 K, r.h. = 11%. The graph has an offset of 0.01 particles cm^{-3}. Initial concentrations were $[O_3] = 4.6 \times 10^{11}$ cm^{-3}, $[SO_2] = 8.1 \times 10^{10}$ cm^{-3}, and [furan] = 1.1×10^{12} cm^{-3}.

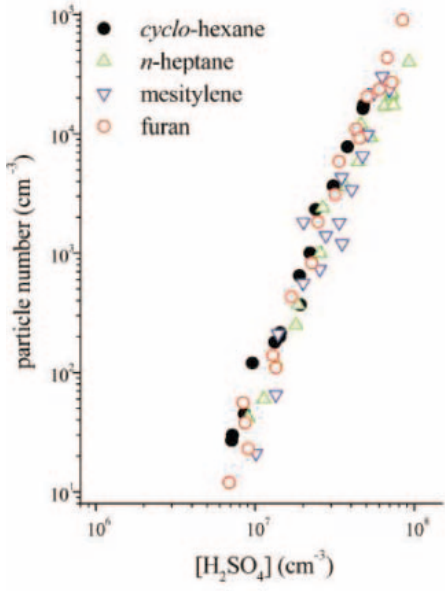

Fig. 2. Experimentally observed particle numbers for different hydrocarbons (*cyclo*-hexane, *n*-heptane, mesitylene, and furan) for OH radical titration, at r.h. = 28%. Initial concentrations of the reactants were $[O_3] = (4.4 \text{ to } 9.8) \times 10^{11}$ cm^{-3}, $[SO_2] = (5.9 \text{ to } 81) \times 10^9$ cm^{-3}, and [HC] = (1.1 to 11) $\times 10^{12}$ cm^{-3}. The amount of converted HC was in the range (7.9 to 31) $\times 10^{10}$ cm^{-3}, and the OH radical concentrations were (8.4 to 18) $\times 10^6$ cm^{-3}.

H_2SO_4 where we observed particle formation using in situ–produced H_2SO_4. Derived particle nucleation rates in the continental atmosphere at ground level are in the range 0.5 to 10 particles cm^{-3} s^{-1} (*27*–*30*).

From the experiments with in situ H_2SO_4 formation, we estimated a nucleation rate of 0.3 to 0.4 particles cm^{-3} s^{-1} for 10^7 cm^{-3} of H_2SO_4 and r.h. = 28% to 49.5% (Fig. 3), using calculated concentration profiles (fig. S1) for defining the nucleation zone (*15*). This finding is in line with the lower limit of the nucleation rates observed in the atmosphere.

Ternary nucleation using H_2SO_4, H_2O, and NH_3 (*9*, *10*) is often assumed in descriptions of new particle formation in the atmosphere. Experimental evidence has been found for the formation of clusters consisting of H_2SO_4 and NH_3 for temperatures up to 285 K, given concentrations of $\sim 10^9$ cm^{-3} for both species (*31*). In our experiment, no NH_3 was added. The measured NH_3 concentrations in the carrier gas were below the detection limit (3.5×10^9 cm^{-3}) of the AiRRmonia system used (*32*). We modeled the NH_3 concentration profile in the tube by taking this value as the initial NH_3 concentration at the entrance of the flow tube and assuming diffusion-controlled wall loss for NH_3 (*33*).

$$NH_3 \rightarrow wall \qquad (4)$$

This modeling results in a maximum NH_3 concentration of $\sim 2 \times 10^8$ cm^{-3} [8 parts per trillion by volume (pptv)] at the beginning of the nucleation zone, dropping substantially with increasing residence time. The modeled average NH_3 concentration in the nucleation zone is below 10^7 cm^{-3} (0.5 pptv). Initial as well as averaged NH_3 concentrations in the nucleation zone are distinctly lower than atmospheric NH_3 mixing ratios measured over continents of 100 to 10,000 pptv (*34*).

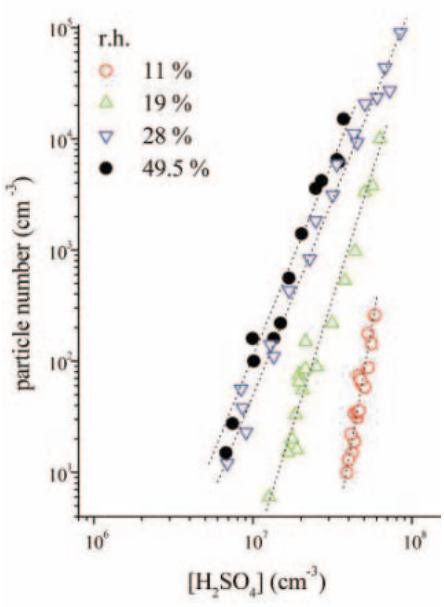

Fig. 3. The number of newly formed particles for r.h. = 11%, 19%, 28%, and 49.5%, given furan for OH radical titration (in situ H_2SO_4 formation in the presence of hydrocarbons).

In the experiments with in situ–produced H_2SO_4 (and in the presence of hydrocarbons), particle formation occurs for $\sim 10^7$ cm^{-3} of H_2SO_4 with a nucleation rate of 0.3 to 0.4 particles cm^{-3} s^{-1} (both parameters comparable to those from the atmosphere), but at distinctly lower NH_3 concentrations than in the atmosphere. For this reason, a substantial role of NH_3 in the nucleation process becomes questionable. Furthermore, the same carrier gas was used in both the experiments with in situ–produced H_2SO_4 and those with H_2SO_4 from a liquid reservoir. This makes NH_3 influence on the barrier H_2SO_4 concentrations needed for nucleation unlikely, because for both experiments, the carrier gas should contain the same unmeasurable amount of NH_3.

We compared our experimental nucleation rates with theoretical ones (*35*, *36*). The H_2SO_4 concentration needed for substantial binary nucleation is $\sim 10^{10}$ cm^{-3}, i.e., far too high compared to the experimental values. Ternary NH_3-influenced nucleation does not explain the observed particle numbers, if NH_3 mixing ratios below 0.5 pptv are assumed (as modeled above). Furthermore, the nucleation rate seems to behave like H_2SO_4 concentration to a power of smaller than 3 to 5, indicating that the nucleation mechanism depends clearly on H_2SO_4 concentration, and suggesting a very close to trimer or dimer (kinetically controlled) nucleation mechanism (*37*).

No definite mechanistic explanation for the different barrier H_2SO_4 concentrations of $\sim 10^7$ cm^{-3} and $\sim 10^{10}$ cm^{-3} can be given. Currently available binary nucleation theories are not able to describe these findings and may not consider the correct nucleation mechanism. Ion-induced nucleation also cannot explain our experimental results, because no ions were present in the experiments. Arguments against NH_3-influenced ternary nucleation include (i) different barrier H_2SO_4 concentrations for nucleation of $\sim 10^7$ cm^{-3} and $\sim 10^{10}$ cm^{-3} despite approximately the same anticipated NH_3 concentration in the carrier gas, and (ii) a too-low NH_3 concentration in the nucleation zone. Tentatively, it can be estimated that the difference in the barrier H_2SO_4 concentration is due to effects connected with freshly produced H_2SO_4 and/or the hydrocarbon's influences on the nucleation and/or the subsequent growth processes. If hydrocarbons bear a meaning for the observed particles, the oxygenated reaction products from pathway 1 (carbonylic substances, etc.) are probably more suitable candidates than their precursor molecules (*14*, *35*). More work is needed to clarify the mechanism leading to the particles observed.

References and Notes

1. C. Wilson, Ed., *Inadvertent Climate Modification, Report of the Study of Man's Impact on Climate* (MIT Press, Cambridge, MA 1971).
2. A. J. Watson, D. C. E. Bakker, A. J. Ridgwell, P. W. Boyd, C. S. Law, *Nature* **407**, 730 (2000).
3. H. R. Pruppacher, J. D. Klett, *Microphysics of Clouds and Precipitation* (Reidel, Dordrecht, Netherlands, 1978).
4. A. Peters, H.-E. Wichmann, T. Tuch, J. Heinrich, J. Heyder, *Am. J. Respir. Crit. Care Med.* **155**, 1376 (1997).
5. G. J. Doyle, *J. Chem. Phys.* **35**, 795 (1961).
6. B. E. Wyslouzil, J. H. Seinfeld, R. C. Flagan, K. Okuyama, *J. Phys. Chem.* **94**, 6842 (1991).
7. Y. Viisanen, M. Kulmala, A. Laaksonen, *J. Chem. Phys.* **107**, 920 (1997).
8. S. M. Ball, D. R. Hanson, F. L. Eisele, P. H. McMurry, *J. Geophys. Res.* **104**, 23709 (1999).
9. D. J. Coffman, D. A. Hegg, *J. Geophys. Res.* **100**, 7147 (1995).
10. P. Korhonen *et al.*, *J. Geophys. Res.* **104**, 26349 (1999).
11. C. D. O'Dowd *et al.*, *Nature* **417**, 632 (2002).
12. C. E. Kolb, *Nature* **417**, 597 (2002).
13. S. H. Lee *et al.*, *Science* **301**, 1886 (2003).
14. R. Zhang *et al.*, *Science* **304**, 1487 (2004).
15. Materials and methods are available as supporting material on *Science* Online.
16. B. J. Finlayson-Pitts, J. N. Pitts Jr., *Chemistry of the Upper and Lower Atmosphere: Theory, Experiments, and Applications* (Academic Press, San Diego, CA, 2000).
17. E. R. Lovejoy, D. R. Hanson, L. G. Huey, *J. Phys. Chem.* **100**, 19911 (1996).
18. J. T. Jayne *et al.*, *J. Phys. Chem.* **101**, 10000 (1997).
19. Here a nearly constant OH radical concentration in the irradiated reaction zone is assumed. This is valid for a constant ratio of OH formation rate to OH consumption rate to be proportional to $[O_3]/[HC]$. Limiting the conversion of O_3 and HC to 10 to 15% makes the assumption of constant OH radical levels viable.
20. R. Atkinson, *Chem. Rev.* **86**, 69 (1986).
21. F. Kramp, S. E. Paulson, *J. Phys. Chem.* **102 A**, 2685 (1998).
22. R. Zellner, *Ber. Bunsenges. Phys. Chem.* **82**, 1172 (1978).
23. D. R. Hanson, F. L. Eisele, *J. Phys. Chem.* **104 A**, 1715 (2000).
24. D. Kashchiev, *J. Chem. Phys.* **76**, 5098 (1982).
25. D. M. Rowley, P. D. Lightfoot, R. Lesclaux, T. J. Wallington, *J. Chem. Soc., Faraday Trans. 1* **87**, 3221 (1991).
26. T. Berndt, O. Böge, F. Stratmann, *Atmos. Environ.* **38**, 2145 (2004).
27. R. J. Weber *et al.*, *Geophys. Res. Lett.* **26**, 307 (1999).
28. W. Birmili, A. Wiedensohler, C. Plass-Dülmer, H. Berresheim, *Geophys. Res. Lett.* **27**, 2205 (2000).
29. W. Birmili *et al.*, *Atmos. Chem. Phys.* **3**, 361 (2003).
30. M. Kulmala *et al.*, *J. Aerosol Sci.* **35**, 143 (2004).
31. D. R. Hanson, F. L. Eisele, *J. Geophys. Res.* **107**, 10.1029/2001JD001100 (2002).
32. J. W. Erisman *et al.*, *Atmos. Environ.* **35**, 1913 (2001).
33. The reactor wall is assumed to be coated with a $H_2O/(H_2SO_4)$ layer. Uptake coefficients for NH_3 on neutral or acidic solutions are in the range 0.05 to 1.0 (*39*), making the assumption of diffusion-controlled wall loss reasonable. The diffusion coefficient was taken from Lechner (*40*).
34. J. H. Seinfeld, S. N. Pandis, *Atmospheric Chemistry and Physics* (Wiley, New York, 1998).
35. H. Vehkamaki *et al.*, *J. Geophys. Res.* **107**, 4622, doi:10.1029/2002JD002184 (2002).
36. I. Napari, M. Noppel, H. Vehkamaki, M. Kulmala, *J. Chem. Phys.* **166**, 4221 (2002).
37. A. A. Lushnikov, M. Kulmala, *Phys. Rev.* **E58**, 3157 (1998).
38. M. Kalberer *et al.*, *Science* **303**, 1659 (2004).
39. E. Swartz *et al.*, *J. Phys. Chem.* **103 A**, 8824 (1999).
40. M. D. Lechner, *Taschenbuch für Chemiker und Physiker* (Springer-Verlag, Berlin, 1992).

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Fig. S1

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Dislocations in Complex Materials

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Deformation of metals and alloys by dislocations gliding between well-separated slip planes is a well-understood process, but most crystal structures do not possess such simple geometric arrangements. Examples are the Laves phases, the most common class of intermetallic compounds and exist with ordered cubic, hexagonal, and rhombohedral structures. These compounds are usually brittle at low temperatures, and transformation from one structure to another is slow. On the basis of geometric and energetic considerations, a dislocation-based mechanism consisting of two shears in different directions on adjacent atomic planes has been used to explain both deformation and phase transformations in this class of materials. We report direct observations made by Z-contrast atomic resolution microscopy of stacking faults and dislocation cores in the Laves phase Cr_2Hf. These results show that this complex dislocation scheme does indeed operate in this material. Knowledge gained of the dislocation core structure will enable improved understanding of deformation mechanisms and phase transformation kinetics in this and other complex structures.

Plastic deformation in a crystal occurs by the processes of slip and twinning. These processes are accomplished by the motion of dislocations whose character is closely related to the structure of the crystal. When these dislocations produce displacements that are less than a unit lattice translation vector in the crystal, they are called partial dislocations and they bound stacking faults. The motion of a partial dislocation can also produce certain types of phase transformations. The partial dislocation associated with the sliding of close-packed planes of atoms over each other during slip, twinning, or shear transformations in face-centered cubic (fcc) metals is the Shockley partial (*1*). In slip, a pair of partial dislocations bounding a stacking fault moves on the slip plane in response to an applied stress to produce plastic deformation. In twinning, a Shockley dislocation sweeps every slip plane, whereas when a Shockley dislocation sweeps alternate slip planes it converts an fcc structure into a hexagonal close-packed (hcp) structure (*1*). Observations on Laves phases (*2*) show that analogous mechanisms could operate in these more complex structures (*3*–*7*). Laves phases, compounds with the AB_2 stoichiometry where the large A atoms and the small B atoms have an ideal radius ratio of 1.225, are the most commonly occurring intermetallic compounds. Cubic, hexagonal, and rhombohedral structures have been imaged with the use of high-resolution electron microscopy (*2*–*4*, *8*), and phase transformation mechanisms based on dislocation motion have been proposed (*2*, *3*, *5*, *9*).

On an atomic level, however, the shearing mechanisms in the Laves phases cannot be identical to those in simple metals because Laves phases are ordered structures with four different atomic planes parallel to the slip plane (*10*). A ball-and-stick model of the hexagonal C14 structure (the Laves phase with the shortest period along the basal plane normal) when viewed along a close-packed direction and with basal planes horizontal is illustrated (Fig. 1A). The alloy Cr_2Hf is used as the example. The structure, like all Laves phases, consists of alternating layers of single small (Cr) atom layers [in the form of a kagome (basket weave) net (*11*)] and three-layer stacks (Hf-Cr-Hf) that contain two low-density planes of large (Hf) atoms separated by a low-density plane of small (Cr) atoms. The great number of possible Laves phases derives from the fact that there are two forms of these three-layer stacks that we designate as **t** and **t′**, which are rotated 180° in the layer plane to each other. In C14, these two arrangements of the three-layer stacks alternate (Fig. 1A) and the stacking sequence of single basal planes (**s**) and triple basal planes (**t**) is **s t s t′**… . All other Laves phases have longer stacking sequences than C14, and thus, during a transformation, some **t** layers must be changed to **t′** and vice versa; this may be accomplished by passage of the transforming Shockley dislocations, much like in the fcc structure.

An ideal technique for viewing the atomic structures in Laves phases is Z-contrast imaging in a high-resolution scanning transmission electron microscope (STEM) (*12*). With its sub-angstrom resolution (*13*), the positions of atom columns are revealed, and, because the atomic number [Z] values for Cr [24] and Hf [72] are very different, the light and heavy atoms may be distinguished. Figure 1B is a micrograph of C14 Cr_2Hf viewed along <11-20> in the same orientation as Fig. 1A.

It is known from conventional TEM observations that the slip planes for the Shockley dislocations in the C14 structure are the basal planes (*4*, *6*), although atomic details remain unresolved. Slip, twinning, and shear transformations are believed, on geometric and energetic grounds, to occur inside the three-layer stack (rather than between the single Cr layer and the three-layer stack) by a mechanism called synchroshear (*3*, *4*, *14*) that consists of two shears in different directions on adjacent atomic planes. The dislocation description of synchroshear requires the passage of two Shockley dislocations through the three-layer stack, one gliding between the lower atom level and the center with one Burgers vector and the other gliding between the center and

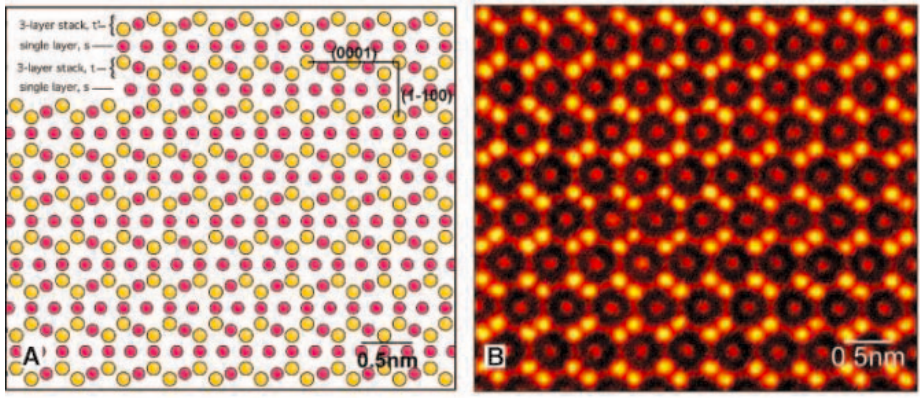

Fig. 1. (**A**) A schematic of the <11-20> projection of the C14 hexagonal structure showing the three-layer stack, **t**, and the 180°-rotated three-layer stack, **t′**, separated by the single layer, **s**. The large atoms (Hf) are in yellow and the small atoms (Cr) are in red. (**B**) A Z-contrast image of the <11-20> projection of the C14 variant of the Cr_2Hf Laves phase. The bright features (yellow) are Hf columns, and the less-intense features (red) are Cr columns.

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the upper level with a different Burgers vector. Figure 2 illustrates the sliding of two rigid blocks, whose faces terminate at the upper and lower Hf layers in the three-layer stack, past each other in response to an applied stress, with an intermediate Cr layer between the two blocks. If it is assumed that the bottom block is stationary, then in response to an applied shear stress the Hf atom in the top block moves in the plane of the paper in the direction shown by the bold black arrow to the position of the Cr atom labeled *X*. For this to happen, however, the Cr atom in the layer between the two rigid blocks needs to get out of the way, and it does so by moving out of the plane of the paper in the direction illustrated by the blue arrow to position *X′*, which was previously occupied by a Hf atom. These two coordinated movements can be described by the motion of two Shockley partial dislocations that for energetic reasons are closely coupled. For this reason, in the literature, the pair of Shockley dislocations has been treated as a single synchro-Shockley partial dislocation with its core spread on two adjacent planes (*9*). The effect of this synchronous translation of the two layers is to convert a **t**-type three-layer stack into a **t′**-type three-layer stack, thereby producing a stacking fault. In Fig. 2, the synchro-Shockley partial dislocation is an edge dislocation.

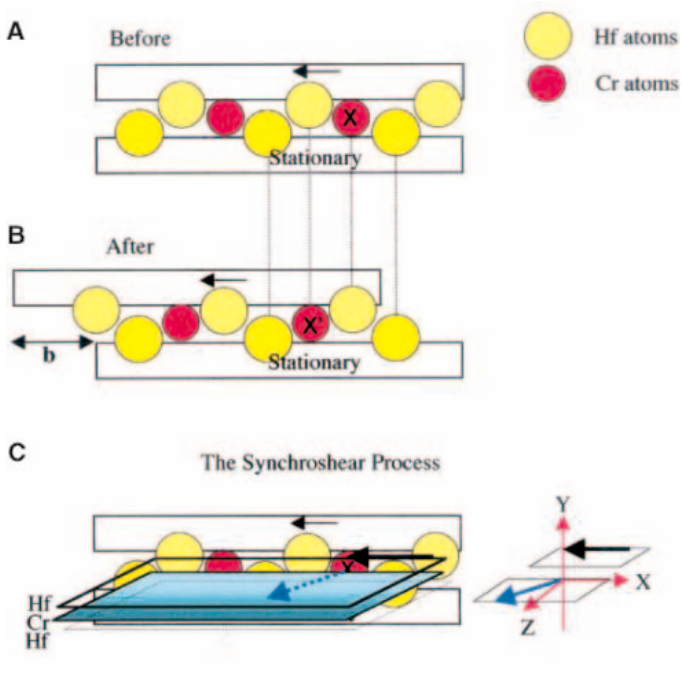

Fig. 2. A schematic illustration of the motion of a synchro-Shockley partial dislocation. (**A**) The initial arrangement of the big (yellow) and small (red) atoms in the three-layer stack, shown with the same orientation as in Fig. 1. The kagome layers above and below the three-layer stack are represented by the rigid blocks, with the bottom block considered stationary and the top block responding to a shear stress by moving to the left. This representation is the motion of a synchro-Shockley partial dislocation with pure edge character. (**B**) The atomic arrangement after synchroshear. (**C**) The synchroshear process is illustrated where all the Hf atoms in the top layer of the three-layer stack move in the plane of the paper to the left by a distance *b* as shown by the bold black arrow, while the Cr atoms move out of the plane of the paper in the middle Cr layer (at 60° to the –*X* direction) as shown by the blue arrow, producing the final configuration shown in (B).

The Z-contrast image in Fig. 3 shows a stacking fault in Cr_2Hf that comes in from the right about halfway up the image (indicated by the arrow) and terminates in the center of the micrograph at the core of a synchro-Shockley dislocation. The construction of a Burgers circuit using the Hf atoms in the triple layer shows closure failure. In the core of the dislocation, a **t** layer (on the left) changes to a **t′** layer (on the right). Relative to the lower Hf atoms, the upper Hf atoms may move either to the right by **b**/2 (where **b** is the Burgers vector), signifying a 30° character for the dislocation, or to the left by **b** (as in Fig. 2), which would imply a pure edge dislocation (90° character). The closure failure of the Burgers circuit in Fig. 3 is 0.15 nm = **b**/2, implying that this dislocation has a 30° character. As may be seen in the figure, the core of the synchro-Shockley dislocation is compact, on the order of 0.5 nm in width and about 0.1 nm high.

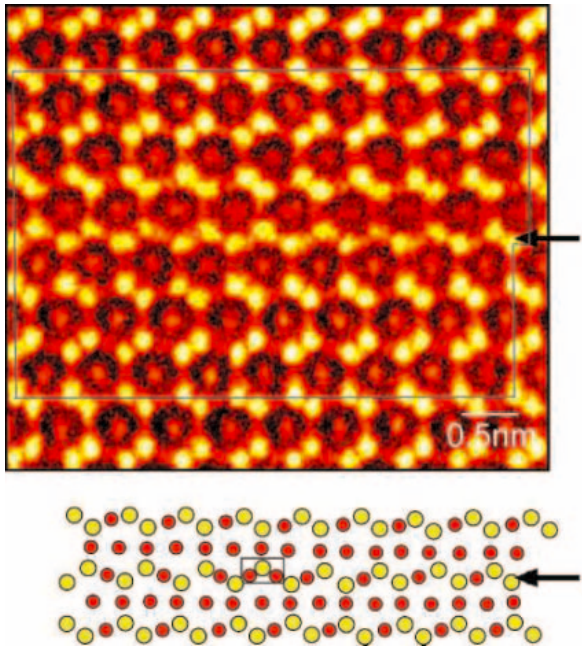

Fig. 3. A Z-contrast image of the <11-20> projection of a synchro-Shockley dislocation bounding a stacking fault in the C14 variant of the Cr_2Hf Laves phase. The fault comes in from the right (indicated by the arrows) and terminates at the dislocation core in the center of the image. The synchro-Shockley partial dislocation in this instance has a 30° character. The Burgers circuit made from Hf atoms failed to close. The schematic below the image, obtained directly from the image, shows a proposed core structure. The indicated Cr columns in the small box in the schematic are included on the basis of the relatively large separation of the adjacent Hf columns.

A Laves phase transformation is illustrated (Fig. 4) where a strip of the cubic C15 Laves phase has formed in the C14 structure by repeated synchroshear. The C15 structure results by synchroshearing the C14 structure on alternate three-layer stacks; this is analogous to ordinary Shockley partial dislocations transforming a hcp structure to a fcc structure, by gliding on alternate close-packed planes. The stacking sequence in Fig. 4 is **....st′stst′st′st′st′st′stst′....** instead of the unsheared **...st′stst′stst′stst′stst′...**.

The concept of synchroshear described above was originally introduced by Kronberg (*14*) to explain the deformation behavior of α-Al_2O_3, although recent results (*15–17*) have confirmed that that is not the case. The Z contrast images of faults and dislocations presented here, however, demonstrate unambiguously that synchroshear [originally proposed by Allen, Delavignette, and Amelinckx (*4*) for Laves phases] actually occurs in the Cr_2Hf Laves phase. Because all Laves phases are constructed from the same building blocks, there is good reason to believe that synchroshear occurs in the others as well. The motion of a single type of defect, a synchro-Shockley partial dislocation, is thought to be responsible for shear phase transformations as well as three mechanisms of deformation: slip, twinning, and stress-induced transformations. In this case, the

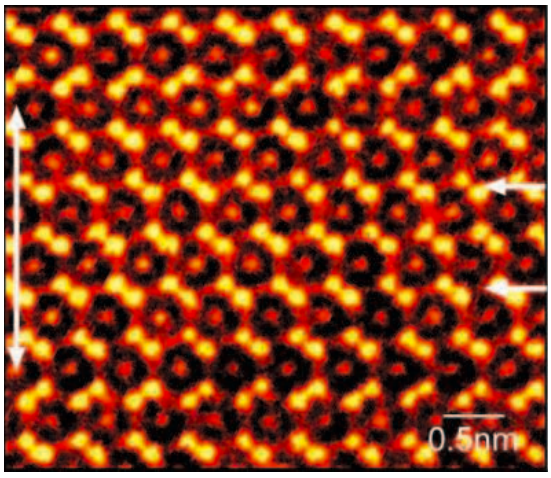

Fig. 4. A Z-contrast image of the <11-20> projection of a faulted region of the C14 variant of the Cr_2Hf Laves phase. This region (between the double arrow on the left) has the C15 cubic structure and could be formed by stacking faults in the C14 structure (indicated by the two short arrows on the right) that result from the passage of two synchro-Shockley dislocations.

kinetics of all four of these processes is controlled by the mobility of synchro-Shockley dislocations. Much understanding could be gained from atomistic simulations of the motion of this type of dislocation. Understanding the structure of the dislocation core and its influence on the ability of Laves phases to deform is central to designing alloys with optimized mechanical properties. In addition, these concepts can be extended to other crystal structures where the slip planes have more than one spacing.

References and Notes

1. J. P. Hirth, J. Lothe, *Theory of Dislocations* (McGraw-Hill Series in Materials Science and Engineering, McGraw-Hill, New York, 1968).
2. F. Laves, H. Witte, *Metallwirtsch. Metallwiss. Metalltech.* **15**, 840 (1936).
3. J. D. Livingston, *Phys. Status Solidi A* **131**, 415 (1992).
4. C. W. Allen, P. Delavignette, S. Amelinckx, *Phys. Status Solidi A* **9**, 237 (1972).
5. Y. Liu, J. D. Livingston, S. M. Allen, *Metall. Mater. Trans. A* **23**, 3303 (1992).
6. K. S. Kumar, D. B. Miracle, *Intermetallics* **2**, 257 (1994).
7. Y. Liu, J. D. Livingston, S. M. Allen, *Met. Mat. Tran. A* **26**, 1441 (1995).
8. Y. Kitano, Y. Komura, H. Kajiwara, E. Watanabe, *Acta Crystallogr. A* **36**, 16 (1980).
9. P. M. Hazzledine, P. Pirouz, *Scripta Metall. Mater.* **28**, 1277 (1993).
10. C. S. Barrett, T. B. Massalski, *Structure of Metals: Crystallographic Methods, Principles, and Data,* vol. 35 of International Series on Materials Science and Technology (Pergamon, Oxford, ed. 3,1987).
11. K. Husimi, *Prog. Theor. Phys.* **5**, 177 (1950).
12. P. D. Nellist, S. J. Pennycook, *Phys. Rev. Lett.* **81**, 4156 (1998).
13. P. D. Nellist *et al.*, *Science* **305**, 1741 (2004).
14. M. L. Kronberg, *Acta Metall.* **5**, 507 (1957).
15. J. B. Bilde-Sørensen *et al.*, *Acta Mater.* **44**, 2145 (1996).
16. P. Pirouz *et al.*, *Acta Mater.* **44**, 2153 (1996).
17. T. Geipel *et al.*, *Acta Mater.* **44**, 2165 (1996).

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End States in One-Dimensional Atom Chains

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End states—the zero-dimensional analogs of the two-dimensional states that occur at a crystal surface—were observed at the ends of one-dimensional atom chains that were self-assembled by depositing gold on the vicinal Si(553) surface. Scanning tunneling spectroscopy measurements of the differential conductance along the chains revealed quantized states in isolated segments with differentiated states forming over end atoms. A comparison to a tight-binding model demonstrated how the formation of electronic end states transforms the density of states and the energy levels within the chains.

The break in translational symmetry at a crystal surface creates surface electronic properties that differ from those in the bulk crystal and localize at the surface layer (*1*, *2*). The formation of surface states and resonances is a general property of solid surfaces, and the delicate interplay between the surface electronic structure and the atomic positions often leads to complex surface reconstructions in which the atoms in the top layer rearrange to minimize the surface energy.

In analogy to the surface of a bulk solid, we expect to observe similar physics in reduced dimensions at the edge or end of a nanostructure. Similar to a two-dimensional (2D) surface state formed at the surface of a bulk sample, an edge or step in a 2D structure breaks the 2D symmetry and can form a 1D edge state (*3*). Likewise, a finite 1D chain of atoms should exhibit zero-dimensional end states at its termini. An end state requires two criteria: (i) The wave function of the state must be localized to the end atoms, and (ii) it must decay exponentially into the chain (*2*). Scanning tunneling microscopy (STM) and scanning tunneling spectroscopy (STS) enable the spatial mapping of differentiated electronic structure within a nanostructure. Thus, the local density of states (DOS) can be mapped in real space, providing direct access to electronic edge or end effects. Furthermore, questions of the spatial variation of the wave function away from the end of a 1D structure can be answered directly.

So far, definitive spectroscopic evidence for the existence of end states in 1D structures has been lacking. Research has instead focused on the quantum mechanics of electrons confined to reduced dimensions within the surface layer. Electrons are partially reflected by step edges on metal surfaces and can exhibit refraction at the interface between two media (*4*–*7*). Electrons also exhibit quantum confinement when trapped within atomic "corrals" assembled by STM (*8*). At the 1D limit, finite atomic chains of Au on NiAl(110) (*9*, *10*) and Cu on Cu(111) (*11*) constructed by STM exhibit quantized electronic states, and the observation of spectroscopic enhancement at or near the ends of these chains suggested the possibility of the formation of end states. However, the weakness of these effects in the systems studied, compounded with the possibility of experimental artifacts (*10*, *11*), precluded unequivocal assignment. In the Si(553)-Au atom chains described below, end states are unequivocally manifested through a marked transfer of the DOS from the empty to the filled states above the end atoms. Furthermore, we found that quantized states within finite chains can no longer be described by a particle-in-a-box model, which has been successful in the previous studies (*9*–*11*), but rather require a model that includes end states.

To fabricate 1D chains, we used the self-assembly of chain reconstructions on stepped

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Si templates driven by the deposition of gold at elevated temperatures (*12*). Gold chain reconstructions on stepped Si have been studied extensively with angle-resolved photoemission (*12*–*16*). Because photoemission gives spatially averaged electronic properties, to date the role of individual defects has remained largely unexplored (*17*, *18*). Here, we focus on Si(553)-Au, which exhibits several metallic bands with 1D dispersion (*19*) and has few defects. In this case, the defects appear as dark voids in STM images (Fig. 1) that leave isolated segments with chain lengths that vary depending on the defect density. All of the measurements were made at room temperature, higher than the temperatures at which charge density wave transitions have been observed in similar 1D chains on Si (*20*, *21*). Such transitions transform the electronic structure from metallic to insulating, so a room-temperature study is desirable to access the chains in their metallic phase.

The formation of end states in finite chain segments is already apparent from comparing STM images of the same sample region taken at opposite polarity (Fig. 1). At a sample bias of +0.5 V (Fig. 1, A, C, and E), the chains appear shorter than at a bias of –1 V (Fig. 1, B, D, and F). This apparent change in length is caused by a contrast reversal over the end atoms, as is apparent from an enlargement of a nine-atom chain (Fig. 1, C and D) and from topography line profiles along the chain (Fig. 1, E and F). At positive bias the end atoms are hardly visible and the atoms second from the end are enhanced, whereas at negative bias the end atoms are enhanced. Such a polarity contrast in STM indicates an underlying difference in the DOS for the empty and filled states near the ends of the chains. On the end atoms, the DOS is transferred from the empty to the filled states.

The differential conductivity as measured by STS provides a direct measure of the local DOS at the tip position and thus allows a detailed study of the electronic variation near the ends of a chain. Figure 2 shows an example for a seven-atom chain with spectra taken beyond the end of the chain, over an end atom, and on an interior atom. Beyond the end of the chain, there is a clear gap with little intensity near the Fermi energy (E_F) (Fig. 2B, green). The boundaries of the gap are consistent with the measured valence-band maximum for Si(553)-Au, –0.3 eV, from photoemission (*12*) and the 1.1-eV band gap of Si. Over the end atom, the differential conductance exhibits a new peak at –0.75 V (Fig. 2B, red). In contrast, the interior atom (Fig. 2B, blue) has an additional peak at +0.5 V inside the band gap.

Spectroscopy along the chains further revealed quantized electronic states that are confined within the isolated chain segments. An isolated atom displays a single state at –0.75 V, which splits into two states at –0.75 and –0.45 V for a two-atom dimer (Fig. 2, C and D). This split is the expected hybridization for a dimer, although the splitting between the states is smaller than in previous studies (*9*–*11*). To best distinguish the two states, we compared spectra from the center and edge of the dimer (Fig. 2D, red). At the center, the state at –0.75 V is clearly seen but the state at –0.45 V appears only as a shoulder. Over the edge of the dimer, although the intensity is low, the –0.75-V peak is absent and allows clear resolution of the –0.45-V state.

In contrast, the three-atom chain exhibits a new peak that is nearly 1 eV higher in energy and is confined to the central atom. This apparent departure for the three-atom chain is attributable to end states, which are characteristic of chains three atoms or more in length. The empty state 0.5 eV above E_F lies in the gap of the Si substrate (Fig. 2B), but states below –0.3 eV are degenerate with the Si bulk bands and are thus technically "end resonances," analogous to the distinction between states and resonances at surfaces of bulk solids.

To elucidate these end effects, we performed spectroscopy in a dense line of points along three-, four-, and five-atom chains (Fig. 3, D, F, and H). These conductance images are formed from individual conductance spectra like those in Fig. 2, B and D, taken at each point along the atom chain. Each spectrum is divided by a second-order polynomial, which approximately removes the transmission function, to allow a more accurate comparison with the theoretical DOS (*22*). This procedure maps spatial variations in the DOS along the chains (*23*). For example, the states seen in the individual spectra for the three-atom chain in Fig. 2D (blue) are mapped in Fig. 3D. The state at +0.5 V (blue circles in Fig. 2D) appears as a dot that is localized to the central atom (position 2 in Fig. 3D). In contrast, the state at –0.7 V (blue triangles in Fig. 2D) has the highest intensity over the end atoms at positions 1 and 3, with lower intensity in the middle. States in the upper corners (>0.8 V) are localized to the regions

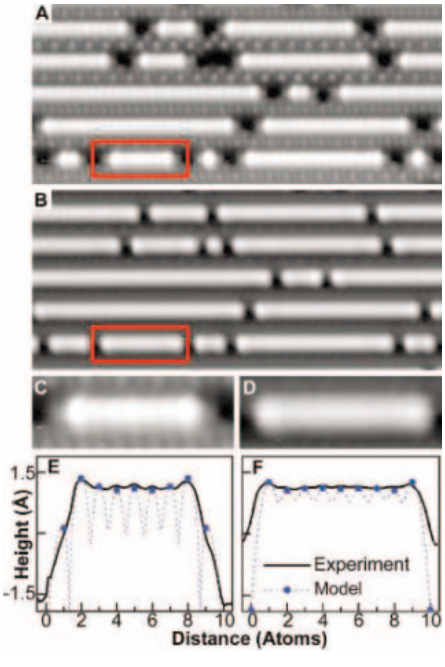

Fig. 1. The effects of end states are visible by comparing STM constant-current mode (0.1 nA) images of the same area (8 by 19 nm) of Si(553)-Au at positive and negative sample biases. The chains appear shorter at +0.5-V sample bias (**A**) than at –1-V sample bias (**B**). The areas inside the red boxes in (A) and (B) are expanded to show an enlargement of a nine-atom chain in (**C**) and (**D**), respectively, and line profiles of the topography along the chain are compared with a tight-binding model in (**E**) and (**F**). The end atoms are barely visible at +0.5 V [(C) and (E)] but are enhanced at –1 V [(D) and (F)].

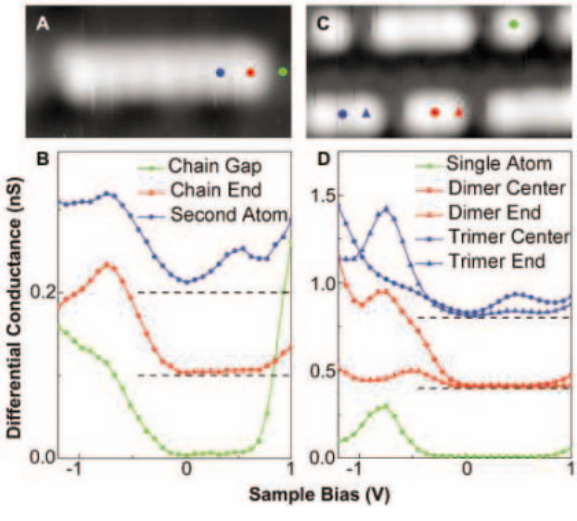

Fig. 2. On the left (**A**), an STM topography image shows a seven-atom chain with (**B**) selected spectra taken in the region beyond the chain (green), over an end atom (red), and over an atom second from the end (blue). On the right (**C**), an STM topography image shows several chains ranging in length from one to four atoms. (**D**) Spectroscopy shows one state for an isolated atom that splits into two states for a dimer, as is expected from the atomic hybridization. The three-atom chain shows a new positive state that is much higher in energy and is localized over the middle atom. The image sizes are (A) 2 by 4 nm and (C) 2.5 by 5 nm. The set point voltages are –1.2 V for (A) and (B) and –0.7 V for (C) and (D). Spectroscopy curves are offset by 0.1 nS in (B) and 0.4 nS in (D) for ease of display and offset baselines for each are also included.

beyond the chain ends and are attributable to either (i) the onset of the conduction band or (ii) empty defect states. The apparent high intensity beyond the ends of the chains is misleading because of the marked inward motion of the tip (>1 Å) that leads to an exponential increase in the differential conductivity (*23*).

To interpret the DOS, we use a tight-binding model in which we consider the states in the chains as atomic wave functions, approximated as a Gaussian at each site, interacting in one dimension. The hopping integral between the wave functions leads to the formation of hybridized states that are a mixture of the atomic wave functions, similar to hybridized states in a molecule. The resulting chain wave functions and eigenenergies are parameterized in terms of the hopping integrals and the binding energies at each atomic site, with the hopping integrals determining the bandwidth and the shape of the band for an infinite chain. The advantage of the tight-binding approach is that it allows differentiation of states at the end atoms. By shifting the electron binding energy on atoms at the ends of the chain relative to the interior atoms, we can model the formation of end states.

A comparison of the DOS for a three-atom chain from the tight-binding calculation (Fig. 3, A, B, and C) and experiment (Fig. 3D) provides the simplest demonstration of the electronic end effects. First, consider the chain without including the end states and with only the nearest-neighbor hopping integral, t_1 = –0.35 eV (Fig. 3A) (*24*). The electronic binding energy determines the energy zero, and the result is similar to a particle in a simple-square well potential with a tight-binding dispersion relation (*11*). The n = 1, 2, and 3 quantum well states have evenly spaced energies and exhibit one, two, and three peaks in the DOS along the chains, respectively. This result contrasts with the experimental data, which has only two resolved states with very different topology (Fig. 3D). The empty states are missing intensity over the end atoms, whereas the filled states have high intensity over the end atoms. Assuming the total DOS at each atomic position is conserved, this result implies a transfer of DOS from the empty to the filled states. Adding a large positive second-nearest-neighbor hopping integral, t_2 = 0.2 eV, to the model effectively lowers the energy of the n = 2 state so it is nearly degenerate with n = 1 (Fig. 3B) but cannot explain the redistribution of DOS over the end atoms. For Fig. 3C, we used different binding energies for the end and interior atoms of –0.6 and 0.27 eV, respectively, with t_1 = –0.19 eV and t_2 = 0.1 eV, which provides the best agreement with the experimental data. The n = 1 and n = 2 states are nearly degenerate and have enhanced DOS over the end atoms. The resulting redistribution of DOS matches qualitatively the DOS in the experiment and explains why only two states are resolved (Fig. 3D). Thus, we can directly attribute the redistribution of DOS to the electronic end effects. In an alternative representation, the transfer of DOS implies a transfer of charge from the interior to the end atoms.

Spectroscopy of longer chains shows similar end states, as is seen for four- and five-atom chains (Fig. 3, E to H). In both cases, the transfer of DOS over the end atoms is similar to that for the three-atom chain, with almost no intensity for the empty states and an accumulation of intensity for the filled states in the range of –0.6 to –0.8 V. Furthermore, similar end states were formed for longer chains (*25*). A tight-binding model with the same few parameters provides a consistent, semiquantitative description of the experimental data for chain lengths ranging from four to nine atoms (*26*). If the model is extended to a semi-infinite chain, the parameters we derived, with binding energy shift $\varepsilon_1 - \varepsilon_0$ = –0.33 eV at the end atom that is comparable to the hopping integral t_1 = –0.34 eV, are consistent with the formation of a localized end state (*2*). The inclusion of second-atom terms in the binding energy shift suggests electronic end effects that extend at least two atoms into the chain. STM topography data from Fig. 1 reflect this assertion and show a clear enhancement of the second atom at positive bias that corresponds to the increased binding energy of the second atoms in the model (*26*).

Our spectroscopy measurements of finite chains also show how end effects can lower the energy of the filled states within the chains, which suggests a possible driving force for the formation of end states. For the four-atom and five-atom chains, the lowest three states are nearly degenerate in energy, with the end effects providing a substantial energy savings (0.4 eV for the filled states in the five-atom chain) when compared with models in which the end effects are omitted. Similar energy savings for the filled states are calculated for all of the chains studied. In contrast, this mechanism is not applicable to previously studied chain systems of Au on NiAl(110) and Cu on Cu(111), which have only empty states (*9*–*11*). The formation of end states does not lower the energy unless they are filled, possibly explaining why a particle-in-a-box model was sufficient in these previous studies. The formation of end states provides a mechanism, in addition to the Peierls distortion (*27*), to lower the total energy for 1D chains of finite length.

From the calculated DOS in the chains, we can model the constant-current STM profiles along the chains and thus verify the origins of the end effects seen at different bias voltages in Fig. 1. Figure 1, E and F, compares calculated and experimental profiles along a nine-atom chain for tunneling into the empty and filled states. The model uses the calculated DOS with the aforementioned parameters (*26*) along with a transmission function that describes the decay of the wave functions into the vacuum. We further assume a constant DOS for the tip and neglect tip convolution effects. The calculated constant-current profiles exhibit pronounced dips between atoms that are much weaker in the experiment because of the finite size of the tip. Nevertheless, the relative heights at the atomic position (filled circles in Fig. 1, E and F) are in fair agreement with the experiment, especially considering the simplicity of the model.

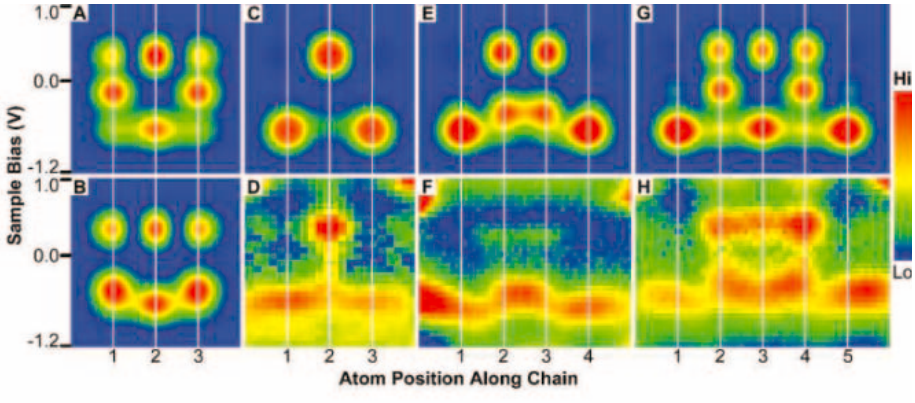

Fig. 3. Tight-binding calculations of the DOS and experimental differential conductance measurements as a function of bias voltage and position along the chain for three-, four- and five-atom chains. The magnitude of the differential conductance is represented on a rainbow color scale, with red as high intensity and blue as low intensity, and plotted as a function of position on the *x* axis and sample bias on the *y* axis. For the three-atom chain, different tight-binding parameters are tested (**A** to **C**) and are compared with an experimental *dI*/*dV* measurement taken with a constant-current set point (**D**). Only the model that includes end states (**C**) agrees with the experiment. The presence of end states is confirmed by extending the model to (**E**) four-atom and (**G**) five-atom chains, providing good agreement with the experimental *dI*/*dV* measurements in (**F**) and (**H**), respectively. The distance along the chains is measured in units of the Si lattice spacing along the chain direction (3.84 Å).

References and Notes

1. A. Zangwill, *Physics at Surfaces* (University Press, Cambridge, 1988).
2. S. G. Davison, M. Stęślicka, *Basic Theory of Surface States* (Oxford Univ. Press, New York, 1992).
3. M. V. Bollinger *et al.*, *Phys. Rev. Lett.* **87**, 196803 (2001).
4. L. Burgi, O. Jeandupeux, A. Hirstein, H. Brune, K. Kern, *Phys. Rev. Lett.* **81**, 5370 (1998).
5. M. F. Crommie, C. P. Lutz, D. M. Eigler, *Nature* **363**, 524 (1993).
6. Y. Hasegawa, P. Avouris, *Phys. Rev. Lett.* **71**, 1071 (1993).
7. J. Repp, G. Meyer, K. H. Rieder, *Phys. Rev. Lett.* **92**, 036803 (2004).
8. M. F. Crommie, C. P. Lutz, D. M. Eigler, *Science* **262**, 218 (1993).
9. N. Nilius, T. M. Wallis, W. Ho, *Science* **297**, 1853 (2002).
10. T. M. Wallis, N. Nilius, W. Ho, *Phys. Rev. Lett.* **89**, 236802 (2002).
11. S. Folsch, P. Hyldgaard, R. Koch, K. H. Ploog, *Phys. Rev. Lett.* **92**, 056803 (2004).
12. J. N. Crain *et al.*, *Phys. Rev. B* **69**, 125401 (2004).
13. J. R. Ahn, H. W. Yeom, H. S. Yoon, I. W. Lyo, *Phys. Rev. Lett.* **91**, 196403 (2003).
14. K. N. Altmann *et al.*, *Phys. Rev. B* **64**, 035406 (2001).
15. R. Losio *et al.*, *Phys. Rev. Lett.* **86**, 4632 (2001).
16. P. Segovia, D. Purdie, M. Hengsberger, Y. Baer, *Nature* **402**, 504 (1999).
17. An STS study of Si(111) 5×2-Au revealed 1D Schottky barriers between insulating and metallic chain segments.
18. H. S. Yoon, S. J. Park, J. E. Lee, C. N. Whang, I. W. Lyo, *Phys. Rev. Lett.* **92**, 096801 (2004).
19. J. N. Crain *et al.*, *Phys. Rev. Lett.* **90**, 176805 (2003).
20. J. R. Ahn, H. W. Yeom, H. S. Yoon, I. W. Lyo, *Phys. Rev. Lett.* **91**, 196403 (2003).
21. H. W. Yeom *et al.*, *Phys. Rev. Lett.* **82**, 4898 (1999).
22. M. M. J. Bischoff, T. Yamada, A. J. Quinn, H. van Kempen, *Surf. Sci.* **501**, 155 (2002).
23. Two caveats must be considered in interpreting any spatially resolved STS measurements. The DOS is convolved with the finite size of the tip leading to a spatial broadening. Also, a constant-current set point determines the tip-sample separation for each measurement, leading to an exponential increase in the differential conductivity when the tip-sample separation decreases, such as off the ends of the chains.
24. Choices with positive t_1 are ruled out from modeling longer chains.
25. J. N. Crain, D. T. Pierce, data not shown.
26. Consistency among different chain lengths requires the inclusion of (i) a nearest-neighbor hopping integral for the end atoms $t_{1\ end} = -0.19$ eV that is reduced as compared with the nearest-neighbor hopping integral between central atoms $t_1 = -0.34$ eV, (ii) a second-nearest-neighbor hopping integral of $t_2 = 0.10$ eV, and (iii) binding energies of $\varepsilon_1 = -0.60$ eV for the end atoms, $\varepsilon_2 = -0.09$ eV for the atoms second from the end, and $\varepsilon_0 = -0.27$ eV for the remaining atoms in the interior of the chain. For the four-atom chain there are no middle atoms, so only energies ε_1 and ε_2 are used.
27. R. F. Peierls, *Quantum Theory of Solids* (Clarendon, Oxford, 1955).

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Photic Zone Euxinia During the Permian-Triassic Superanoxic Event

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Carbon and sulfur isotopic data, together with biomarker and iron speciation analyses of the Hovea-3 core that was drilled in the Perth Basin, Western Australia, indicate that euxinic conditions prevailed in the paleowater column during the Permian-Triassic superanoxic event. Biomarkers diagnostic for anoxygenic photosynthesis by Chlorobiaceae are particularly abundant at the boundary and into the Early Triassic. Similar conditions prevailed in the contemporaneous seas off South China. Our evidence for widespread photic-zone euxinic conditions suggests that sulfide toxicity was a driver of the extinction and a factor in the protracted recovery.

The most severe extinction of the past 500 million years occurred in the Late Permian (*1*, *2*). The biotic crisis was accompanied by an oceanic anoxic event (OAE) that may have lasted up to 8 million years. Although different authors report various anoxic intervals, the most severe conditions persisted during the first 1 to 3 million years (*3*, *4*). Anoxia has been proposed to have had a major role in driving the extinction (*5*, *6*); surface outcropping of sulfidic waters and emissions of hydrogen sulfide to the atmosphere provide a kill mechanism that might account for the terrestrial and marine extinctions (*7*).

In anoxic zones of modern-day stratified lakes or restricted marine environments (e.g., the Black Sea and Antarctic fjords), conditions are favorable for bacterial reduction of sulfate to sulfide (e.g., *8*). Chlorobiaceae (green sulfur bacteria) are typical of these environments in which hydrogen sulfide extends into the photic zone, where it serves as the electron donor required for anoxygenic photosynthesis. Chlorobiaceae use a distinct assemblage of light-harvesting pigments comprising bacteriochlorophylls *c*, *d*, and *e* and the carotenoids isorenieratene and chlorobactene. Identification of these compounds, or their diagenetic alteration products, in sediments provides unequivocal evidence for photic zone euxinic (PZE) conditions in the past (e.g., *9*–*12*).

Here, we use carbon and sulfur isotopic data and biomarker and iron speciation analyses in a drill core (Hovea-3) from the onshore Perth Basin, Western Australia (*13*), to establish the redox conditions in the water column of the southern Tethys Ocean during the Permian-Triassic (P-T) superanoxic event. Biomarkers diagnostic for anoxygenic photosynthesis by Chlorobiaceae were identified in P-T boundary sediments of the organic-matter (OM)–rich Hovea-3 core and in coeval samples from the OM-lean Meishan-1, a new core drilled at the type section of Meishan, South China (fig. S1); these biomarkers demonstrate that waters of the Tethys Ocean were periodically euxinic in the photic zone during and after the extinction event.

Changhsingian and Griesbachian sediment samples of Hovea-3 (1960- to 1995-m depth) contain C_{18} and C_{19} aryl isoprenoids (Figs. 1A and 2A), and the Griesbachian sediments contain isorenieratane, the C_{40} parent hydrocarbon (Figs. 1A and 2B and fig. S2). Highly specific bacteriochlorophylls can also give rise to distinctive maleimides (*9*). Methyl *iso*-butyl maleimide was identified in the polar fractions [see (*13*) for separation of maleimides]. The highest concentrations of all these pigment derivatives are preserved in the Griesbachian, reflecting high green sulfur bacterial activity and, thus, PZE conditions. Isorenieratane and aryl isoprenoids (including low pristane/phytane ratios) also occur in the latest Changhsingian and earliest Induan (Griesbachian) sediments (beds 22 to 27) of the global boundary stratotype section and point (GSSP) at Meishan, South China, which suggests that PZE conditions were widespread (Fig. 1B and Fig. 3).

Although the above data provide evidence for PZE during the P-T transition, the presence of benthic epifaunal macroinvertebrates such as *Claraia* and spirorbids dem-

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onstrates that, because these animals would have required some oxygen, the euxinia was episodic (*14*).

Independent evidence for euxinic conditions in the Lower Triassic seas off Australia is provided by high amounts of metalloporphyrins, in particular vanadyl (VO) porphyrins (Fig. 2C) (*15*). These samples also contained high concentrations of Ni (II) porphyrins.

We also measured iron species, dithionite-extractable (Fe_D), pyrite (Fe_P), and total iron (Fe_T) (*16*) in the Hovea-3 sediments. ($Fe_D + Fe_P$)/Fe_T values up to 0.7 indicate euxinic conditions in the basal-most Triassic (Fig. 2D), comparable to values reported from the modern euxinic Black Sea (*17*). Variations in the ratio may be due to changes in the oxycline position, the area of sediment covered by anoxic waters, and changes in weathering rates.

The isotopic composition of the oceanic sulfur reservoir is partly controlled by the balance of bacterial sulfate reduction and sulfide oxidation. These processes lead to ^{34}S-depleted sulfide and related pyrite (*18*) and may result in sulfate relatively enriched in ^{34}S. Burial of pyrite thus removes isotopically light sulfur from the seawater pool. Sedimentary sulfides in the Hovea-3 samples (Fig. 2E) are depleted in ^{34}S compared with contemporaneous seawater sulfate (*19*, *20*) and follow a trend toward heavier values approaching the P-T transition, as seen in carbonate-associated sulfate in other P-T samples from northern Italy and Iran (*19*, *20*).

Sulfur-isotope fractionations between sedimentary pyrite and contemporary sulfate (*19*, *20*) up to about 50 to 60 per mil (‰) indicate that reservoir effects did not substantially influence the isotopic signatures. The $^{34}S/^{32}S$ ratios for the sulfides in the Basal Triassic of the Perth Basin are consistent with euxinic conditions as found in the modern Black Sea and the Pliocene Mediterranean Basin (*21*). The change in isotope discrimination implies that there was a perturbation in the sulfur cycle in the Upper Permian and a relative increase in the fraction of sulfur buried as pyrite in the Lower Triassic compared with the Permian (*22*) or that there was a change in other factors (e.g., quality of OM) influencing overall sulfur isotope discrimination. Isotope shifts in reduced sulfur across the P-T boundary indicate changes in the sulfur cycle similar to those reported in sections from Japan (*22*).

Stable carbon isotopic data from the bulk kerogen fraction of Hovea-3 record an abrupt 7.5‰ negative shift from the Upper Permian to the Lower Triassic consistent with a localized palynofacies change from charcoal-wood dominated OM to algal-amorphous OM, respectively (*23*). In contrast, $\delta^{13}C$ values of the molecular fossils pristane and phytane vary gradually across the P-T transition (Fig. 2F), representing in part a change in OM inputs or an increase in stratification toward the Triassic that caused enhanced recycling of ^{13}C-depleted CO_2. However, recent $\delta^{13}C$ data on higher plant and phytoplankton biomarkers show similar isotopic changes across the P-T transition, which indicates a global disruption of the carbon cycle (*24*).

The $\delta^{13}C$ values of pristane and phytane, derived from phytol side chains of the chlorophylls of algae and cyanobacteria, are robust proxies for the isotopic composition of phytoplankton (e.g., *25*, *26*). In contrast, C_{14}-C_{18} *n*-alkyl carbon chains have many inputs, comprising primary producers and heterotrophs, and their $\delta^{13}C$ values represent a weighted average of these. If derived from primary sources such as algae and cyanobacteria, they should be depleted in ^{13}C compared with the co-occurring isoprenoids by 1.5‰ (e.g., *25*, *26*). This pattern is observed in the Triassic data (Fig. 4), in which there is independent evidence, such as the abundance of porphyrins and algal microfossils, for high primary productivity. Alternatively, isotopic enrichment of C_{14}-C_{18} *n*-alkyl carbon chains can occur through heterotrophic processing of primary photosynthate or dom-

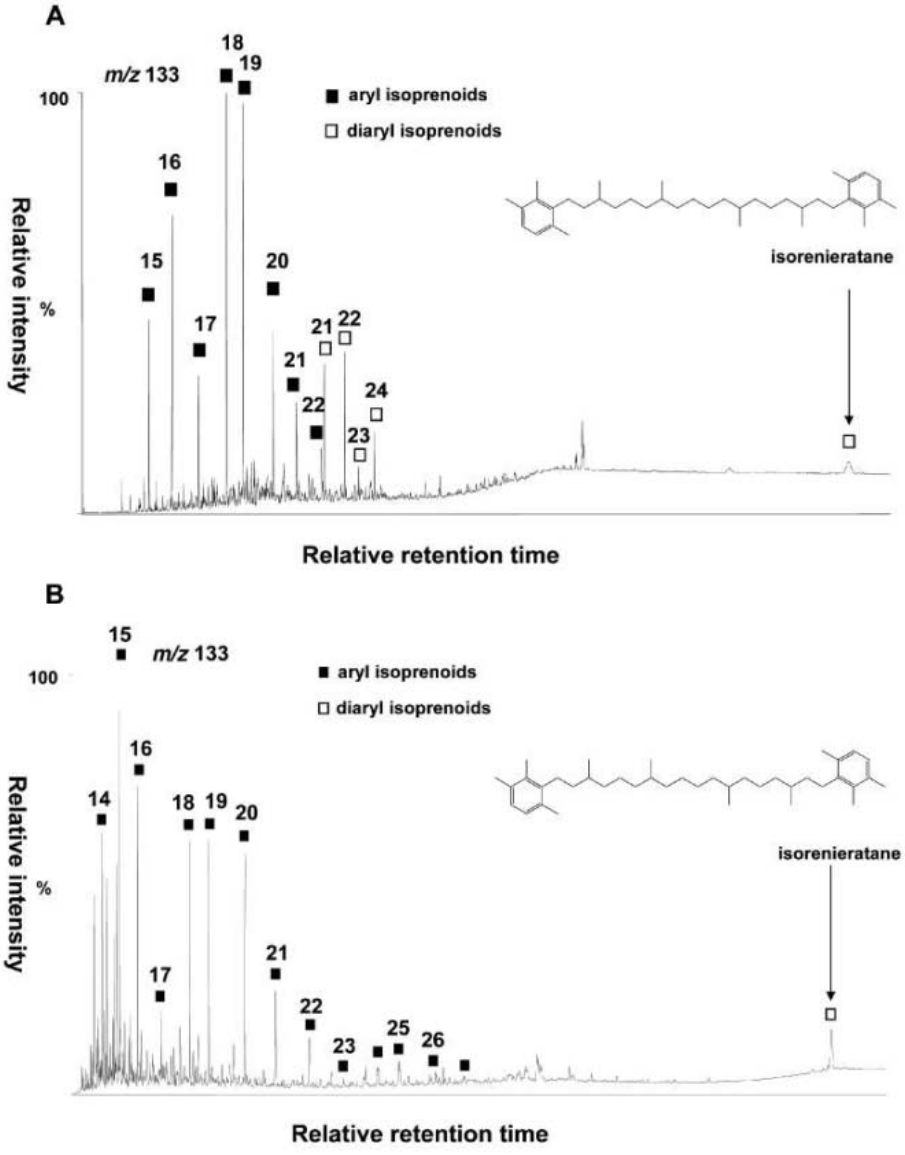

Fig. 1. Typical gas chromatography–mass spectrometry selected-ion chromatogram for the 133-Da fragment ion that is diagnostic for aryl isoprenoids of an aromatic hydrocarbon fraction [see (*14*) for fractionation of extracts]. (**A**) From 1979.9 m (Hovea-3). This trace shows pseudohomologous series C_{12}-C_{31} compounds identified as aryl isoprenoids with a 2,3,6-trimethyl substitution pattern and the C_{40} biomarker isorenieratane. These hydrocarbons were identified by comparison with a reference sample (fig. S2). (**B**) From 107.94 m (Meishan-1). This trace for Meishan Bed 24-6 shows an assemblage of Chlorobiaceae biomarkers similar to those seen in Hovea-3. The enhanced relative abundance of isorenieratane reflects a lower degree of thermal maturity in the sediments from South China.

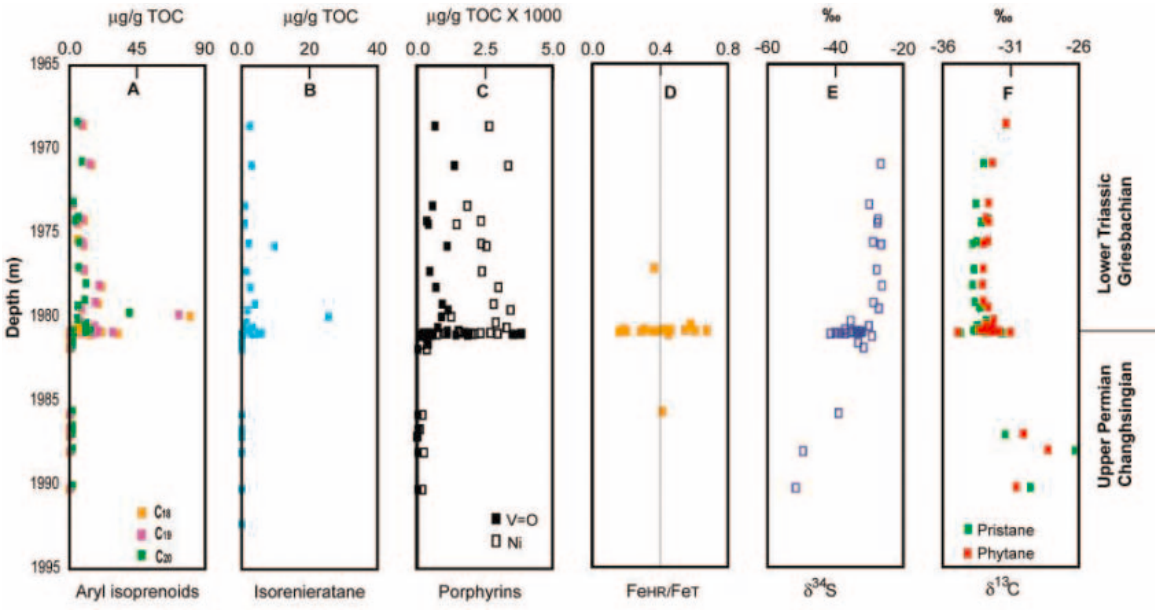

Fig. 2. Composite plot of Hovea-3 organic and inorganic data. (**A**) Aryl isoprenoid concentrations (µg/g of total organic carbon). (**B**) Isorenieratane concentrations (µg/g of total organic carbon). (**C**) Concentrations of vanadyl and nickel porphyrins (µg/g of total organic carbon) [see (*14*) for analysis of porphyrins]. (**D**) Fe_{HR}/Fe_T ratios. Fe_{HR}, highly reactive iron; Fe_T, total iron; Fe_p, pyritic iron [see (*14*) for isolation of iron species]. (**E**) $\delta^{34}S$ of pyrite relative to Vienna Canyon Diablo troilite (VCDT) standard in ‰ [see (*14*) for sulfur isotope analyses]. (**F**) $\delta^{13}C$ of pristane and phytane relative to Vienna Pee Dee belemnite (VPDB) standard in ‰ [see (*14*) for 5A molecular sieving and compound-specific isotope analyses].

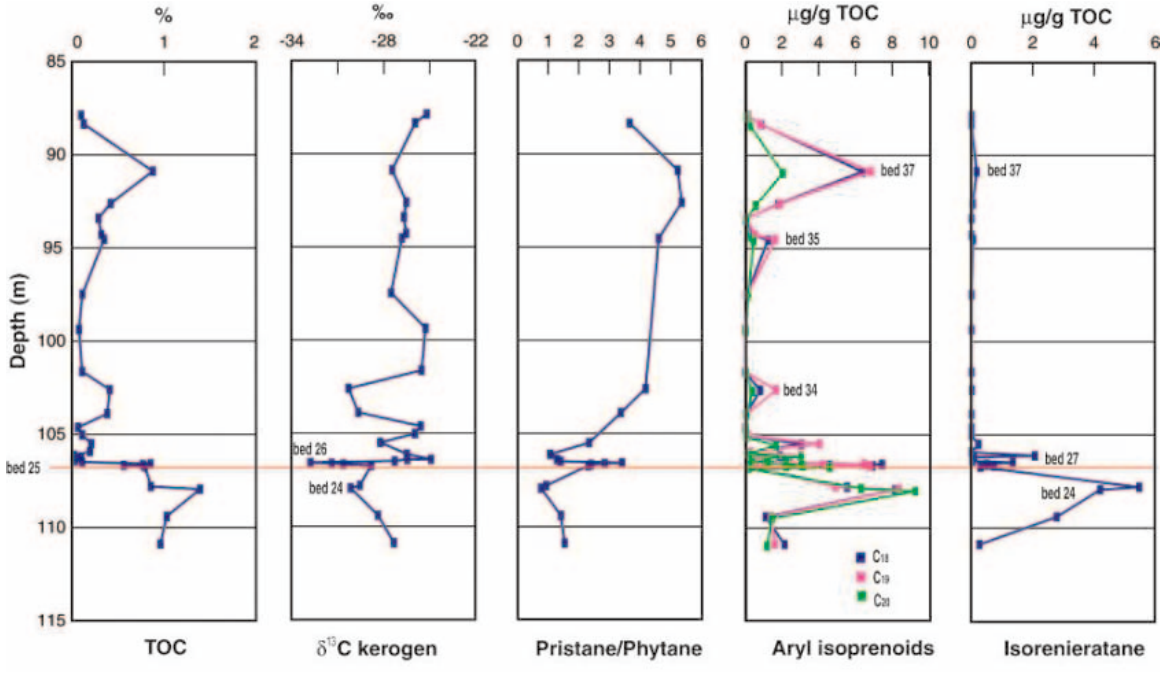

Fig. 3. Composite plot of Meishan-1 organic geochemical, biomarker, and bulk carbon isotopic data. Pristane/phytane ratios show their lowest values just below and just above the extreme negative carbon-isotopic excursion. Maxima of aryl isoprenoid and isorenieratane abundances in beds 24 to 27 bracket the extinction horizon. Notably, there are additional maxima of these markers at beds 34, 35, and 37, which indicates that there was a waxing and waning of PZE conditions that continued well into the Triassic (fig. S1).

inant inputs of isotopically heavy bacterial biomass. This pattern of anomalous isotopic ordering is seen in the Permian sediments. Figure 4 demonstrates this phenomenon, which suggests that there was a shift in the mode of carbon cycling at the P-T transition in Hovea-3. A similar reversal in the expected C-isotopic relation between polymethylenic and polyisoprenoid lipids has been reported for the Proterozoic-Cambrian transition (*27*). Moreover, this transition in C-isotopic relations is also evident in data from the P-T transition of Western Slovenia (*28*), where the isotopic trends of inorganic carbon and kerogen are similar to those seen at Meishan and the Southern Alps. Together, these data provide further evidence for a switch in the mode or extent of organic carbon remineralization at the P-T transition.

The data show that PZE conditions occurred during the P-T superanoxic event and document a major disruption of the carbon and sulfur cycles. The onset of PZE in Hovea-3 coincides with a sharp facies change, reflecting rapid transgression (e.g., *23*). The association of oxygen-poor water and rapid transgression is key for the hypothesis that anoxia caused the extinction event (*5*). Given that similar conditions prevailed elsewhere in the Tethys Ocean during the P-T event, we propose that sulfide toxicity in the ocean and emissions of hydrogen sulfide to the atmosphere were important drivers of the largest mass extinction in the past 500 million years and may have also been a factor in the protracted recovery (*7*).

The local association of PZE and high-sedimentary total organic carbon contents (TOC) at Hovea-3 are similar to those of other Mesozoic OAEs (*29*). However, the conditions creating the record here were not repeated elsewhere, even within Western

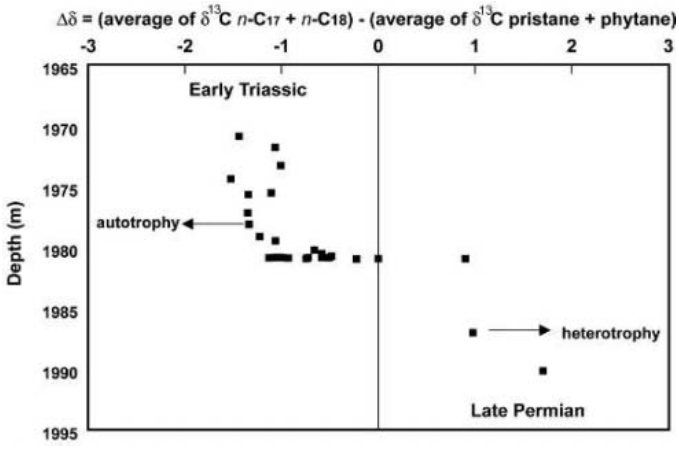

Fig. 4. $\Delta\delta$ = (average $\delta^{13}C$ of n-C_{17} and n-C_{18}) – (average $\delta^{13}C$ of pristane and phytane) ‰ (relative to VPDB standard in ‰) with depth (meters). Measurements determined by isotope-ratio-monitoring gas chromatography–mass spectrometry (IsoPrime, Micromass, Manchester, UK) of saturate, branched/cyclic, and n-alkane fractions [see (*14*) for 5A molecular sieving and compound-specific isotope analyses].

Australia, and must have been very localized. Because PZE also occurred in the contemporaneous seas off South China, localized high algal productivity probably played a key role in the formation of a petroleum-rich source rock in the Perth Basin.

References and Notes

1. D. Erwin, *Nature* **367**, 231 (1994).
2. M. J. Benton, R. J. Twitchett, *Trends Ecol. Evol.* **18**, 358 (2003).
3. P. B. Wignall, R. J. Twitchett, *Science* **272**, 1155 (1996).
4. Y. Isozaki, *Science* **276**, 235 (1997).
5. P. B. Wignall, A. Hallam, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **93**, 21 (1992).
6. A. H. Knoll, R. K. Bambach, D. E. Canfield, J. P. Grotzinger, *Science* **273**, 452 (1996).
7. L. R. Kump, A. Pavlov, M. Arthur, Y. Kato, A. Riccardi, *Abstracts of GSA Annual Meeting*, Paper 81-6 (Geological Society of America, Seattle, WA, 2–5 November 2003).
8. T. Trull *et al.*, *EOS Trans. Am. Geophys. Union* **82**, 306 (2001).
9. K. Grice *et al.*, *Geochim. Cosmochim. Acta* **60**, 3913 (1996).
10. R. E. Summons, T. G. Powell, *Geochim. Cosmochim. Acta* **51**, 557 (1987).
11. M. Koopmans *et al.*, *Geochim. Cosmochim. Acta* **60**, 4467 (1996).
12. K. Grice, P. Schaeffer, L. Schwark, J. R. Maxwell, *Org. Geochem.* **25**, 131 (1996).
13. Materials and methods are available as supporting material on *Science* Online.
14. B. M. Thomas *et al.*, *Aust. J. Earth Sci.* **51**, 423 (2004).
15. Sedimentary porphyrins provide a link to precursor chlorophylls in photosynthetic organisms indicative of primary production in paleowaters. Ni/VO porphyrin ratios are indicators of redox conditions (*30*).
16. $Fe_{HR} = Fe_D + Fe_P$ to Fe_T is an indicator of water-column redox conditions in ancient/modern environments (*17*, *31*). $Fe_{HR}/Fe_T > 0.4$ are typical for euxinic settings with pyrite formation in the water column. Fe_{HR}/Fe_T values < 0.4 are found in sediments below oxic bottom waters, where pyrite precipitates in the sediment. Fe_{HR}/Fe_T ratios < 0.4 do not exclude deposition in a euxinic environment but may indicate changes in the source/depositional setting (*17*).
17. T. F. Anderson, R. Raiswell, *Am. J. Sci.* **304**, 203 (2004).
18. D. E. Canfield, A. Teske, *Nature* **382**, 127 (1996).
19. R. J. Newton, E. L. Pevitt, P. B. Wignall, S. H. Bottrell, *Earth Planet. Sci. Lett.* **218**, 331 (2004).
20. C. Korte *et al.*, *Int. J. Earth Sci.* **93**, 565 (2004).
21. H. F. Passier *et al.*, *Nature* **397**, 146 (1999).
22. Y. Kajiwara, S. Yamakita, K. Ishida, H. Ishiga, A. Imia, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **111**, 367 (1994).
23. C. B. Foster, G. A. Logan, R. E. Summons, J. D. Gorter, D. S. Edwards, *Aust. Pet. Prod. Explor. Assoc. J.* **37**, 472 (1997).
24. K. Grice *et al.*, unpublished data.
25. J. M. Hayes, *Rev. Mineral. Geochem.* **43**, 225 (2001).
26. S. Schouten *et al.*, *Geochim. Cosmochim. Acta* **62**, 1397 (1998).
27. G. A. Logan, J. M. Hayes, G. B. Hieshima, R. E. Summons, *Nature* **376**, 53 (1995).
28. V. Schwab, J. E. Spangenburg, *Appl. Geochem.* **19**, 55 (2004).
29. R. D. Pancost *et al.*, *J. Geol. Soc. London* **161**, 353 (2004).
30. M. D. Lewan, J. B. Maynard, *Geochim. Cosmochim. Acta* **46**, 2547 (1982).
31. Y. Shen, A. H. Knoll, M. R. Walter, *Nature* **423**, 632 (2003).

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Materials and Methods
Figs. S1 and S2
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Abrupt and Gradual Extinction Among Late Permian Land Vertebrates in the Karoo Basin, South Africa

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The Karoo basin of South Africa exposes a succession of Upper Permian to Lower Triassic terrestrial strata containing abundant terrestrial vertebrate fossils. Paleomagnetic/magnetostratigraphic and carbon-isotope data allow sections to be correlated across the basin. With this stratigraphy, the vertebrate fossil data show a gradual extinction in the Upper Permian punctuated by an enhanced extinction pulse at the Permian-Triassic boundary interval, particularly among the dicynodont therapsids, coinciding with negative carbon-isotope anomalies.

The Permian extinction is universally portrayed as the most catastrophic of all Phanerozoic mass extinctions (*1*), yet its cause remains problematic. Various hypotheses include climate change due to increased atmospheric CO_2 and/or CH_4 (*2*), the effects of extraterrestrial impact (*3*–*5*), the effects of the eruption of the Siberian Traps, and some synergistic combination of these (*6*), among others. An important test of any mechanism is a consideration of the pattern of extinctions. The marine extinctions are well described in several areas, notably Meishan, China (*7*). Here, we report new chemostratigraphic, biostratigraphic, and magnetostratigraphic data from multiple stratigraphic sections located in the Karoo basin of South Africa that provide an exceptionally detailed record of the terrestrial extinctions.

Permian-Triassic (P-T) strata in the central Karoo basin provide the most intensively investigated record of vertebrate fossils from

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the Upper Permian through the Triassic (*8*). These strata are dominantly fluvial overbank sediments deposited near the center of a subsiding retroarc foreland basin (*9*). We have sampled across 200 m of the Palingkloof Member of the Balfour Formation and the overlying Katberg Formation, where we recognize four units spanning the Upper Permian and Lower Triassic (*10*). Fossils were collected from these strata at five different areas. Hence, correlating the sections, which is difficult between fluvial sections and has been notably problematic in the Karoo (*11*), is critical.

To correlate the stratigraphy, we obtained a magnetostratigraphic record (Fig. 1) (*12*) from three sections [some data are also in (*13*)]. Samples from Unit II and Unit III are all of normal polarity (Chron N1), and we identified a reversed polarity magnetozone (R1) ~5 m beneath the Unit I-II contact (*14*). At Lootsberg and Wapadsberg, the normal-polarity zone extends well up into the Katberg Formation (Unit IV). At Lootsberg, where the youngest strata were sampled of all the sections, there is a change to reversed polarity (R2) above about 130 m and a final switch back to normal (N2) at the top. Given paleontological constraints (*8*), we correlate the long normal found in Units II, III, and most of IV with magnetozone SN1 of the classic German Trias sections (*15*) and thus define the top and bottom of the Griesbachian stage in the Karoo. The superjacent reversal at Lootsberg Pass is most parsimoniously correlated with magnetozone SR1 of central Germany, R2 of the Southern Alps, and GR1 of the Canadian Arctic. Although there is some uncertainty about the reversal pattern near the P-T boundary (*16*), recent records in Europe suggest that the boundary occurs just above the base of a reversed-to-normal-polarity transition in Germany, although there has been a recent report placing it slightly lower, in the uppermost part of the reversed chron (*17*). The base of Unit II is thus approximately coincident with the P-T boundary.

These correlations were tested by measuring sedimentary carbon isotope ($\delta^{13}C_{carb}$ and $\delta^{13}C_{org}$) stratigraphy at all the sections at meter or submeter sampling frequency (*12*) (Fig. 2), a finer resolution than has been done before (*18*). This earlier P-T isotope study in the Karoo found a single negative $\delta^{13}C_{carb}$ excursion approximately coincident with the last occurrence of the latest Permian

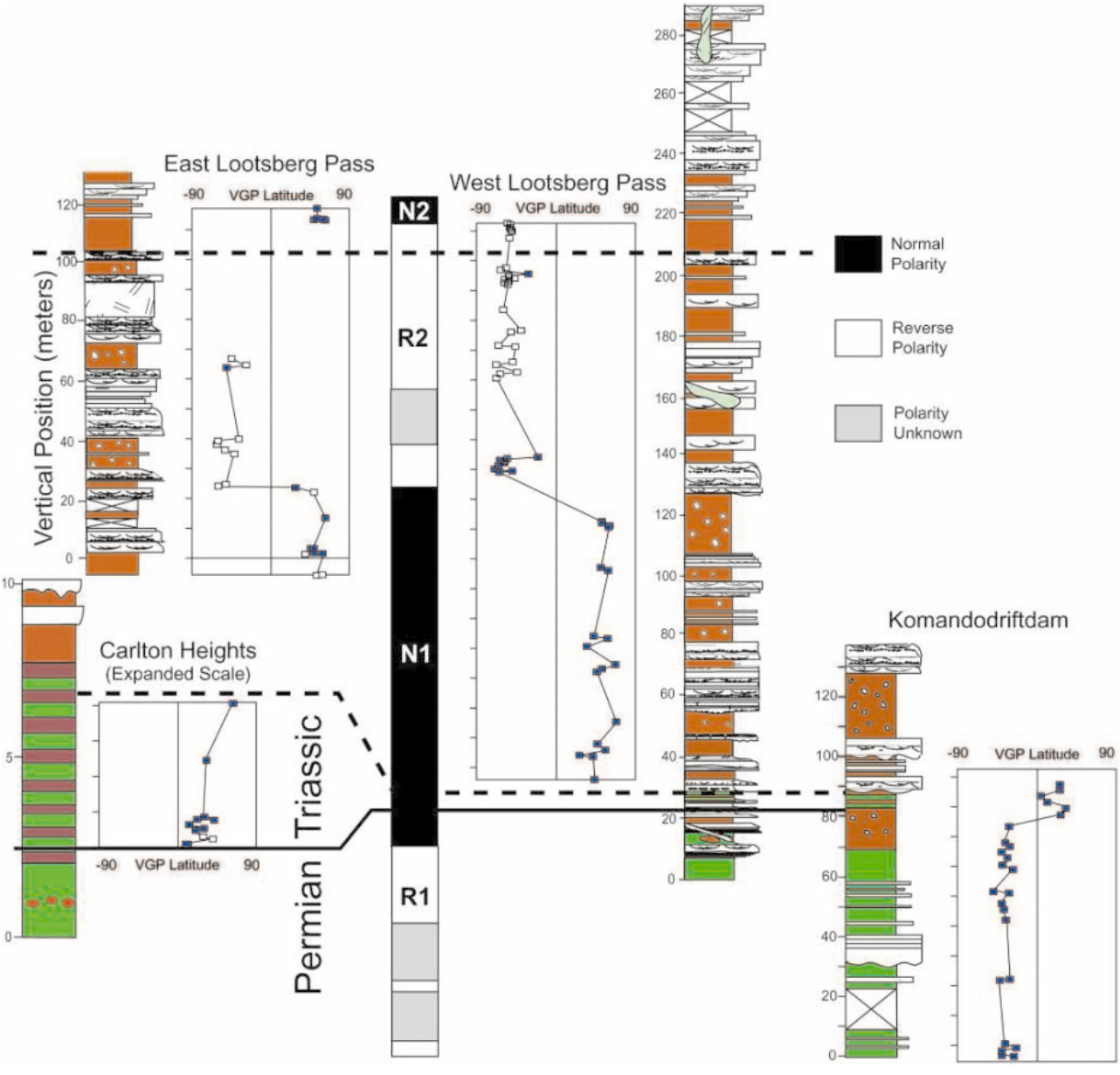

Fig. 1. Magnetostratigraphic correlation across the Karoo basin. The dashed lines represent the correlated positions between the stratigraphic sections.

zonal index fossil *Dicynodon*. The global $\delta^{13}C$ record for the P-T boundary period is known in varying detail from several dozen marine and fewer nonmarine sites (e.g., *19, 20*). Intensively sampled sections show a gradual decline in the sedimentary $^{13}C:^{12}C$ ratio upward through the Upper Permian, then a sudden decline of >2‰ at or near the paleontologically defined extinction boundary, followed by a gradual increase in $\delta^{13}C$. The sudden $\delta^{13}C$ decline precedes the formal P-T boundary based on the first occurrence of the Triassic conodont *Hindeodus parvus* at the global stratotype section and point (GSSP) P-T stratotype in Meishan, China, where the boundary has been placed at Bed 27. At Meishan, the lowest $\delta^{13}C$ values are found in Bed 25 (*7*), coincident with the level of maximal species disappearance (94% loss of then-extant marine invertebrates). Thus, the mass extinction and sharp negative excursion in $\delta^{13}C$ are slightly older than the formal stratigraphic boundary.

We sampled both bulk sediment and carbonate paleosol nodules for carbon isotope stratigraphy from our measured sections. Soil carbonate $\delta^{13}C$ ($\delta^{13}C_{carb}$) is a function of both the $\delta^{13}C$ of atmospheric CO_2 ($\delta^{13}C_{CO2}$) and the partial pressure of CO_2 in the atmosphere (pCO_2), and thus soil $\delta^{13}C_{carb}$ records can be more scattered than marine $\delta^{13}C_{carb}$ records (*21*). Nevertheless, the $\delta^{13}C_{carb}$ results show a prominent drop within Unit I that is consistent with our assessment that Unit I/Unit II contact marks the P-T extinction (Fig. 2). The $\delta^{13}C_{carb}$ values maintain a broad minimum in Unit II, a pattern similar to global marine $\delta^{13}C_{carb}$ records. The lowermost negative excursion in $\delta^{13}C_{carb}$ observed in the Karoo most parsimoniously correlates with the singular, lower Griesbachian negative $\delta^{13}C_{carb}$ excursion reported recently from a Late Permian–Late Triassic carbonate platform in the Nanpanjiang Basin, Guizhou Province, China (*22*). Furthermore, the broad swings in $\delta^{13}C_{carb}$ values that were measured higher in the Karoo sections (Fig. 2) appear to be consistent with Smithian and Spathian age $\delta^{13}C_{carb}$ excursions in the same marine $\delta^{13}C_{carb}$ record.

The bulk sedimentary organic carbon isotope records ($\delta^{13}C_{org}$) from the Carlton Heights and Lootsberg sections provide further support for these conclusions (Fig. 2). The data also supports the magnetostratigraphic correlation of Unit II between the northern (Carlton Heights and Bethulie regions) and southern (Lootsberg and Wapadsberg regions) parts of the basin. At the Carlton Heights and Lootsberg sections, $\delta^{13}C_{org}$ decreases from ~ –24‰ Vienna Pee Dee belemnite (VPDB) to ~ –26‰ VPDB across the uppermost meter of Unit I and remains there through Unit II and into Unit III. Between 15 m and 22 m (Unit III), $\delta^{13}C_{org}$ increases in both sections to ~ –21‰ and then decreases ~1‰ at the base of Unit IV. This pattern is typical of P-T $\delta^{13}C$ records measured elsewhere in the world. The Wapadsberg and Bethulie $\delta^{13}C_{org}$ records do not show any substantial negative excursions, particularly not at the P-T boundary, but both of these sections are extensively intruded by Mesozoic dolerite dikes and sills. We suggest that this igneous activity has homogenized the primary $\delta^{13}C_{org}$ record at these two sections.

Fossil vertebrate biostratigraphy confirms that Unit II is essentially contemporaneous across the Karoo basin. At all sections, the highest occurrence of the uppermost Permian zonal index, *Dicynodon lacerticeps*, is found either immediately below (at the fossiliferous Lootsberg, Wapadsberg, and Bethulie sections) or at most several meters below the base of Unit II (at the fossil-poor Carlton Heights and Kommandodrift Dam sections). *Dicynodon lacerticeps* was never found in or above Unit II, and the first Triassic fossil common to all sections—*Lystrosaurus* sp. C—was found in the lower strata of Unit III but never in Unit II.

In summary, three independent correlation methods support our contention that Unit II is both essentially isochronous across the Karoo basin and also time equivalent with the P-T boundary in China. This allows us to use this unit as a datum surface against which our vertebrate range taxa can be plotted and to compare the patterns of extinction with those observed at other P-T sections.

Over a period of 7 years, we collected 126 skulls assigned to 21 vertebrate taxa from the sections shown here (reptile or amphibian) (Fig. 3) (*23*). We treat these taxa as species, although further study will probably result in an even greater number of taxa. We found 13 taxa in Unit I (Upper Permian), only 4 of which persist into Unit II, and 12 taxa in Units II to IV. Six of the 13 taxa

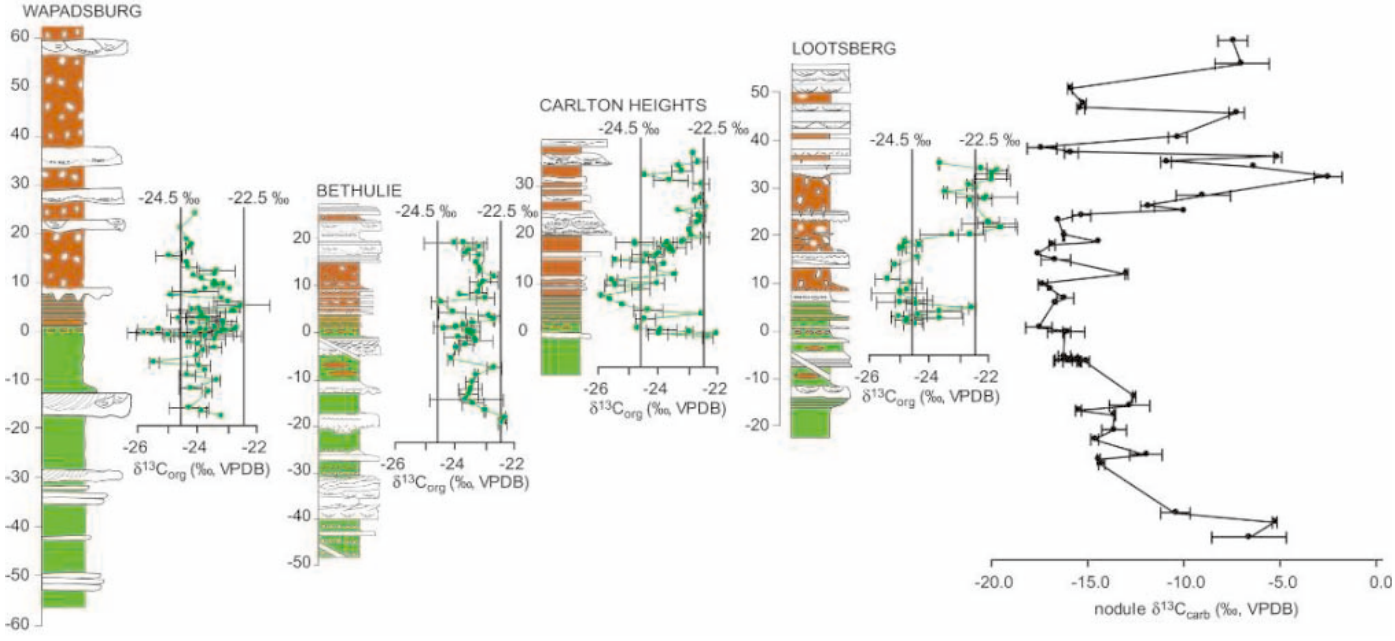

Fig. 2. The combined soil nodule $\delta^{13}C_{carb}$ record and individual lithologic and $\delta^{13}C_{org}$ records for four sections in the Karoo basin. The two sections at left (Wapadsburg, southern basin; Bethulie, northern basin) have been strongly affected by heating, as evidenced by a lack of primary paleomagnetic signal from either section. The results from Carlton Heights and Lootsberg Pass, however, have similar negative $\delta^{13}C_{org}$ excursions within Unit II, followed by an increase in $\delta^{13}C_{org}$ values in Units III and IV. The $\delta^{13}C_{carb}$ curve at the far right is combined data from carbonate nodules obtained from the Carlton Heights and the Lootsberg regions.

found in Unit I show their last appearance datums (LADs) in the last 10 m of Unit I, which suggests an enhanced rate of extinction in the latest Permian. These range terminations occurred either before or simultaneously with the reduced $\delta^{13}C_{org}$ values toward the top of Unit I.

Incomplete preservation or collection failure can make abrupt extinctions look gradual, i.e., the Signor-Lipps effect (*24*, *25*), so the assumption of simultaneous extinction of the included taxa was tested using the Kolmogorov-Smirnov (K-S) goodness-of-fit test (*26*). The case for the Karoo basin is complicated, because some of our sampled taxa (i.e., *Pristerodon* sp. and *Aelurognathus* sp.) might have become extinct before the base of Unit II or long after (*Ictidosuchoides* sp., *Thrinaxodon* sp., *Lystrosaurus* sp. C.), thereby complicating the statistical protocols (*27*). Thus, we calculated confidence intervals on stratigraphic ranges (*28*). We chose 20% confidence intervals following the improved method for testing extinction levels (*29*) for each of the nine taxa (Table 1).

Four taxa (*Theriognathus* sp., *Dicynodon lacerticeps*, *Lystrosaurus* sp. A, and *Rubidgea* sp.) appear to have become extinct at or near the base of Unit II (Fig. 3). We are also confident that *Pristerodon*, *Aelurognathus*, and the abundant and widespread taxon *Diictodon* sp. were extinct before deposition of Unit II, whereas the taxa *Lystrosaurus* sp. B, *Moschorhinus* sp., and *Owenetta* sp. became extinct during, or soon after, the initiation of sedimentation marking the Katberg Formation (Unit IV) (Fig. 3). The 90% confidence interval on the position of the mass extinction extends from 0 m (stippled level A) to about 20 m, or from the base of Unit II up into the middle part of Unit III. Thus, there is a low probability that the mass extinction of the Karoo vertebrates occurred within the Katberg Formation (stippled level B), where the mass extinction has been traditionally placed (*30*).

We have also used confidence intervals to examine the origination of taxa. Theoretically, if the majority of Permian extinctions occurred at a specific horizon, we would expect to observe, after the extinction, a follow-on origination of new species into vacated niches (or immigration into the basin). We do not observe this. By inverting the procedure for fossil disappearances, we calculated 20% range extensions downward and from this computed a 94% probability confidence interval for origination (Fig. 3). The results suggest it is probable that at least some species originated before the deposition of Unit II, whereas others originated during and after Unit II deposition. The distribution of originating taxa fails the K-S test for random distribution of new taxa at the 99% level, suggesting that Triassic taxa origination was in response to some event that occurred before the end of the Permian. We caution that new taxa origination may violate the assumption of equal probability of preservation, because new taxa are likely to be rare and uncommon. This effect may overestimate the calculated ranges for originations.

A further caveat is that we are implicitly accepting an empty-niche model brought about by the extinction, even though some or many niches may have been reconstructed

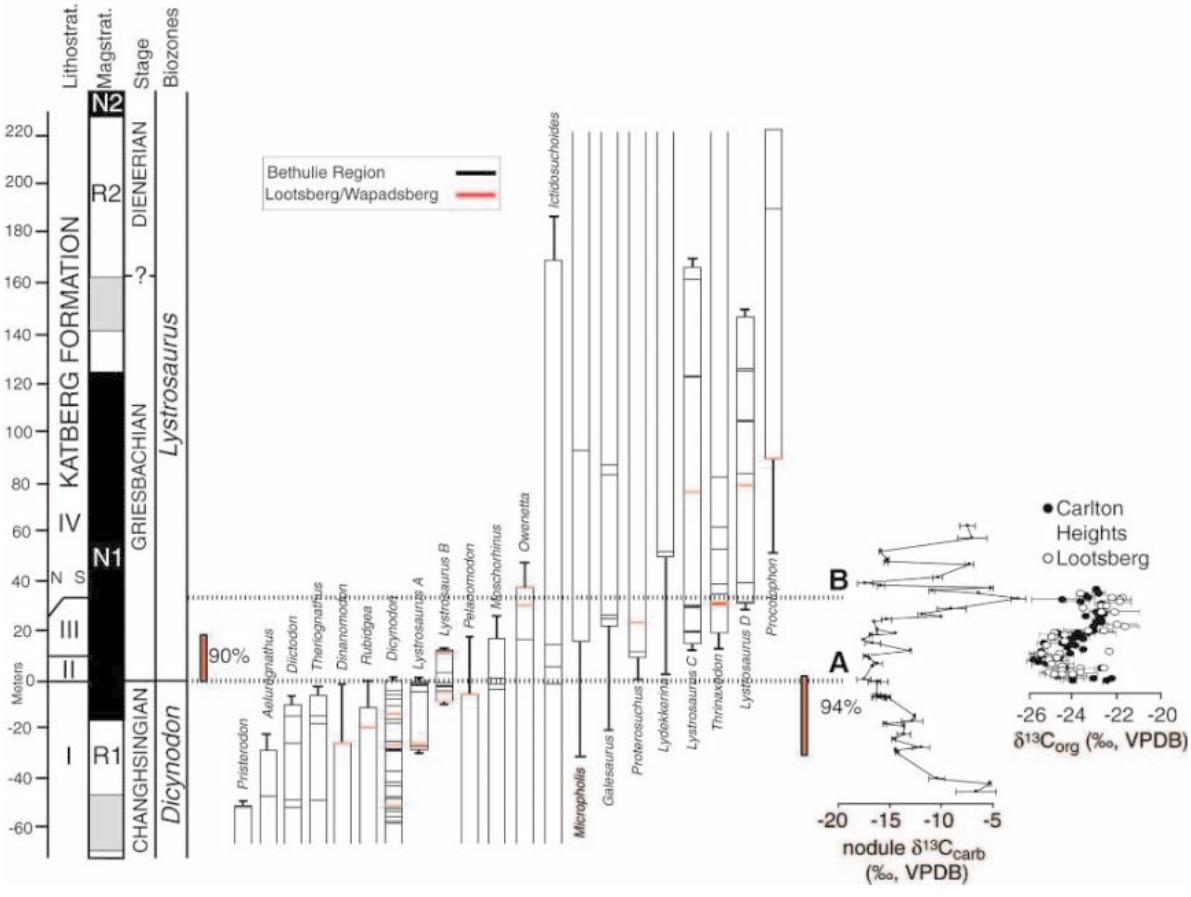

Fig. 3. Magnetostratigraphic, biostratigraphic, and carbon isotopic records from the Karoo basin. This figure can be compared with the generic range chart for the entire Permian/Lower Triassic sequence in the study area (*12*). The red bar on the left is the 90% confidence interval for Permian taxa extinction; the red bar on the right is the 94% confidence interval for Triassic taxa origination, assuming that originations were in response to catastrophic extinction. Level A is the base of Unit II, which is correlated to the highest occurrence of Permian index fossil *Dicynodon lacerticeps*. Level B is the base of Unit IV (Katberg Formation), where a spike in fungal spore abundance and the P-T boundary have been recorded previously (*32*). Of particular note is the appearance of *Lystrosaur* A and *Lystrosaur* B below the P-T boundary. *Lystrosaurs* are Triassic animals, and the appearance of two species below the P-T boundary is evidence that major evolutionary changes were under way before the end of the Permian.

and not refilled. The pattern of extinction and origination appears to be consistent with an enhanced rate of extinction coincident with or just below Unit II, but the number of extinct species there may be no more than five (eight species become extinct in Unit I). The most thorough compilation of the vertebrate record from the *Dicynodon* Zone in the Karoo (*8*) shows that 10 of 21 taxa present at the base of the ~300-m-thick zone are absent in its upper third, indicating that considerable extinction is occurring throughout the zone and not just at its top (*31*). This observation can be supported by replotting known vertebrate ranges in the Karoo such that last occurrences are sequential (*12*), based on the new data presented here and range data from lower zones (*8*). The resulting figure (fig. S4) shows what appears to be a change from an approximately constant background extinction (as recognized by the number of taxa disappearing per unit thickness of strata) below the uppermost Permian, with enhanced extinction in the *Dicynodon* Zone culminating at the P-T extinction pulse at the top of Unit I. In the Lower Triassic–aged *Lystrosaurus* Zone, there is a reduced extinction rate high in Unit II and in Unit III. Two patterns are thus apparent: gradual extinction leading up to the P-T and a pulse of even higher extinction marking the boundary. This pattern is visible both at the species level (Fig. 3) and at the generic level (fig. S4) and is also observed at Meishan among invertebrate marine fossils.

We thus suggest that factors other than the sudden extinction of taxa stimulated the origination or appearance (through emigration) of new Triassic species into the basin. Our statistical inference (and the discovery of *Lystrosaurus* sp. A and B) that Triassic vertebrate fauna may have predated the main Permian extinction pulse is unlike the pattern of mammalian radiation after the Cretaceous-Paleogene (K-P) extinction, the only clear example of a mass extinction associated with a major impact event. The pattern that we observe for the P-T is consistent with a long-term deterioration of the terrestrial ecosystem, with a heightened pulse of both extinction and origination approximately coincident with the P-T boundary.

Unfortunately, the ranges of Permian and Triassic fossil plant remains in our study sections add little information about the pattern of extinction in the Karoo basin or about the relative timing of extinction among plants and animals there. A recent palynological study at Carlton Heights (*32*) identified a spike in fungal spore abundance at the base of our Unit IV (the Katberg Formation) and claimed the fungal spike to be the top of the *Dicynodon* Zone and thus the P-T boundary. However, the study provided insufficient information to ascertain whether the base of Unit IV is Permian or Triassic in age based on palynomorphs (*33*), and we have found in all of our sections, including the site in question, that the Katburg Formation begins well above the top of the Permian.

We interpret the biostratigraphic data presented here as consistent with a period of environmental stress during the latest Permian in this basin, punctuated by a short interval of even greater perturbation, i.e., a long "press" with one (or more) pulses superimposed. A single proximal cause might explain the extinction patterns, such as long-term environmental degradation having reached a critical threshold that triggered a short-term extinction event through ecosystem collapse. Alternatively, the long- and short-term effects observed in the Karoo could have two (or more) different causes. We searched for evidence of an end-Permian bolide impact, such as the impact clays and ejecta layers found commonly in the environmentally similar Hell Creek Formation (Late Cretaceous), Montana, which are associated with the Chicxulub K-P impact. Neither our search nor previous searches in the Karoo sections for minerals associated with large-body impact (*34*) have met with success, although fluvial facies can contain hiatuses and the absence of impact evidence must be tempered with caution.

The P-T southern Karoo basin and the K-P Hell Creek Formation strata were deposited by similar fluvial systems, and they can be compared. The Hell Creek vertebrate paleontological record is constantly diverse up through the last Cretaceous zone, followed by a catastrophic extinction coeval with a negative excursion in the $\delta^{13}C$ record (*35*, *36*) and clear sedimentological and mineralogical evidence of a large-body impact; $\delta^{13}C$ returns to pre-event values within a narrow stratigraphic interval (*35*). The Karoo record is entirely different, and we conclude that if an impact occurred at all, it had a minor role in the end-Permian extinctions in the Karoo. The geologic data from the Karoo are consistent with a more protracted catastrophic ecosystem collapse than a sudden impact would produce.

Table 1. Confidence that vertebrates became extinct or originated prior to deposition of lithological Unit II by application of the equation $C = 1 - (G/R + 1)^{-(H-1)}$, where G is the interval between the highest occurrence of the taxon and the base of Unit II, R is the taxon's observed stratigraphic range, and H is the number of fossiliferous strata within the range of R (*28*). The null hypothesis of a random distribution of fossil horizons is rejected. Taxon confidence (C), extinction before deposition of Unit II. Confidence, origination before deposition of Unit II.

Taxon	Confidence (C) (extinction before deposition of Unit II)	Confidence (origination before deposition of Unit II)
Pristerodon sp.	0.875	
Aelurognathus sp.	0.5	
Diictodon sp.	0.52	
Theriognathus sp.	0.31	
Rubidgea sp.	0.2	
Dicynodon lacerticeps	0.17	
Lystrosaurus sp. *A*	0.13	
Lystrosaurus sp. *B*	0	
Pelanomodon sp.	.05	
Moschorhinus sp.	0	
Owenetta sp.	0	
Ictidosuchoides sp.	0	
Micropholis sp.		0.08
Galesaurus sp.		0.2
Proterosuchus sp.		0.25
Lydekkerina sp.		0.21
Lystrosaurus sp. C		0.69
Micropholis sp.		0.08
Thrinaxodon sp.		0.55
Procolophon sp.		0.54

References and Notes

1. D. H. Erwin, *Nature* **367**, 231 (1994).
2. A. Knoll *et al.*, *Science* **273**, 452 (1996).
3. L. Becker *et al.*, *Science* **291**, 1530 (2001).
4. A. R. Basu *et al.*, *Science* **302**, 1388 (2003).
5. L. Becker *et al.*, *Science* **304**, 1469 (2004).
6. M. J. Benton, R. J. Twitchett, *Trends Ecol. Evol.* **18**, 358 (2003).
7. Y. G. Jin *et al.*, *Science* **289**, 432 (2000).
8. B. Rubidge, *Geol. Surv. S. Afr. Biostratigraphy* **1**, (1995).
9. O. Catuneanu *et al.*, *Basin Res.* **10**, 417 (1998).
10. Two sections were sampled near Lootsberg Pass (S31, 51.005; W24, 52.299, and S31, 49.334; W24, 48.565), one section near Wapadsberg Pass (S31, 52.474; W24, 54.882), one section near Carlton Heights (S30, 35.425; W25, 439.135), one section near Kommandodrift Dam (S31, 76.506; W24, 49.980), and two sections near Bethulie (S30, 24.989; W26, 17.234, and S30, 26.675; W26, 18.006). Four lithostratigraphic facies are present: Unit I, dark gray to gray mudstones, siltstones, and sandstones with sedimentary structures typical of meandering river deposits; strata show rubification in the uppermost meters. Unit II, 3- to 5-m-thick, rhythmically bedded laminated mudrock, described as an event bed (*37*). Unit III, red concretionary mudstone and thin sandstone. Unit IV (Katberg Formation), thick olive-green sandstone with conglomeratic bases interbedded with thinner red siltsone and mudstone; sandstones have sedimentary structures typical of braided river deposits.

11. G. Retallack *et al.*, *Geol. Soc. Am. Bull.* **115**, 1133 (2003).
12. Materials and methods are available as supporting material on *Science* Online.
13. M. O. de Kock, J. L. Kirschvink, *Gond. Res.* **7**, 175 (2004).
14. All samples passed tests for baked contact, class B reversal, and magnetostratigraphic consistency. The reversal in the upper part of Lootsberg Pass was corroborated at a second parallel section ~1 km to the east, where a reversal of similar thickness was found at the same stratigraphic horizon. We have used this pattern to correlate and subdivide the Katberg Formation across the Karoo basin.
15. M. Szurlies *et al.*, *Earth Planet. Sci. Lett.* **212**, 263 (2003).
16. M. Steiner *et al.*, *J. Geophys. Res.* **94**, 7343 (1989).
17. J. Nawrocki, *Terra Nova* **16**, 139 (2004).
18. K. G. MacLeod *et al.*, *Geology* **24**, 227 (2000).
19. M. J. De Wit *et al.*, *J. Geol.* **110**, 227 (2002).
20. W. T. Holser, M. Margaritz, *Geochim. Cosmochim. Acta* **56**, 3297 (1992).
21. T. E. Cerling, *Global Biogeochem. Cycles* **6**, 307 (1992).
22. J. L. Payne, *Science* **305**, 506 (2004).
23. The actual P-T boundary is defined by the base of the Triassic system or the first appearance of the conodont *H. parvus* in marine strata. The base of the Triassic cannot be identified in the Karoo until a terrestrial index fossil is formally chosen. At present, we have placed the P-T boundary at the level of the highest Permian taxon, a practice that runs contrary to accepted stratigraphic procedure. Here, each taxon is treated as a species; we realize that taxonomic study of each is required. Pending the formal systematic treatment of *Lystrosaurus* (*38*), we designate the four separate species of *Lystrosaurus* from our study area as *Lystrosaurus* sp. A, B, C, and D.
24. P. W. Signor III, J. H. Lipps, *Geol. Soc. Am. Spec. Pap.* **190**, 291 (1982).
25. Preservational biases may also have controlled the observed pattern of extinction. However, because there are more fossils in Units III and IV than in the upper 30 m of Unit I, we would expect to find the Permian taxa if they continued higher in the section. As that is not the case, we conclude that the observed ranges are real samples of the preservable fauna in the depositional basin.
26. M. S. Springer, *Paleobiology* **16**, 512 (1990).
27. A. Solow, *Paleobiology* **29**, 181 (2003).
28. C. R. Marshall, *Paleobiology* **20**, 459 (1994).
29. S. Wang, C. R. Marshall, *Paleobiology* **30**, 5 (2004).
30. G. H. Groenwald, J. W. Kitching, *Geol. Surv. S. Afr. Biostratigraphy* **1**, 35 (1995).
31. G. M. King, *S. Afr. Mus. Sidney Haughton Mem. Lect.* **3**, 1 (1990).
32. M. B. Steiner *et al.*, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **194**, 405 (2003).
33. There has been much recent criticism of the so-called P-T fungal spike as a chronostratigraphic marker, based on the discovery of multiple horizons at some localities and the complete absence of the marker at others, most notably at the important Greenland locality (*39*), as well as the possibility that the fossils may not have come from fungi at all (*40*).
34. L. Coney *et al.*, *L.P.S.C.* **35**, 1488 (2004).
35. N. C. Arens, A. H. Jahren, *Palaios* **15**, 4 (2000).
36. P. Sheehan *et al.*, *Science* **254**, 835 (1991).
37. P. D. Ward *et al.*, *Science* **289**, 1740 (2000).
38. J. Botha, R. Smith, in preparation.
39. R. J. Twitchett *et al.*, *Geology* **29**, 351 (2001).
40. C. Foster, M. Stephenson, *First Int. Conf. Palynology, Abst.* **57** (2002).

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Figs. S1 to S4
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Aconitase Couples Metabolic Regulation to Mitochondrial DNA Maintenance

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Mitochondrial DNA (mtDNA) is essential for cells to maintain respiratory competency and is inherited as a protein-DNA complex called the nucleoid. We have identified 22 mtDNA-associated proteins in yeast, among which is mitochondrial aconitase (Aco1p). We show that this Krebs-cycle enzyme is essential for mtDNA maintenance independent of its catalytic activity. Regulation of *ACO1* expression by the HAP and retrograde metabolic signaling pathways directly affects mtDNA maintenance. When constitutively expressed, Aco1p can replace the mtDNA packaging function of the high-mobility-group protein Abf2p. Thus, Aco1p may integrate metabolic signals and mtDNA maintenance.

Mitochondrial DNA (mtDNA) nucleoids have been purified from several organisms (*1*–*5*). In addition to DNA packaging proteins, which are required for mtDNA maintenance (*6*, *7*), nucleoids also contain proteins whose functions are ostensibly unrelated to mtDNA activities (*1*, *5*, *8*, *9*). We previously identified 11 proteins that are associated with mtDNA (*1*) (Table 1) and have now identified 11 more (whose gene names are indicated in bold in Table 1). These proteins can be grouped into four functional categories: (I) mtDNA transactions with no other known functions in mitochondria; (II) protein import and mitochondrial biogenesis; (III) the citric acid cycle and upstream glycolytic steps; and (IV) amino acid metabolism.

As proteins in category III could potentially connect respiratory and fermentative metabolism to mtDNA maintenance, we examined mtDNA stability in strains in which a selection of these genes were inactivated. Expression of some category III genes is repressed by glucose (*10*). Therefore, mutant cells were grown in raffinose, a fermentable carbon source that does not repress mitochondrial respiration, and then assayed for the fraction of respiratory-deficient (petite) mutants in the population. mtDNA is relatively stable in *kgd1*Δ and *kgd2*Δ cells, less so in the *pda1*Δ, *pdb1*Δ, *idh1*Δ, and *lpd1*Δ strains, and very unstable in *aco1*Δ cells (Fig. 1A). mtDNA was previously noted to be unstable when some of these genes were inactivated (*11*). Further experiments established that *ACO1* is essential for mtDNA maintenance. Southern blot analysis of total cellular DNA from nascent meiotic segregants derived from an *aco1*Δ/*ACO1* ρ^+ diploid strain showed that the *aco1*Δ spores lack mtDNA (Fig. 1B).

Aco1p, citrate synthase (CS), and two subunits of nicotinamide adenine dinucleotide (NAD^+)–dependent isocitrate dehydrogenase (collectively, the aconitase metabolon) function to produce α-ketoglutarate, a precursor to glutamate. Expression of the genes encoding these proteins is positively regulated by the glucose-repressible HAP2-5 transcription complex and the transcription factors Rtg1p and Rtg3p (*12*), which are components of the mitochondria-to-nucleus retrograde (RTG) signaling pathway (*13*). Aco1p localizes to the mitochondrial matrix, as revealed by fluorescence microscopy of an Aco1p–green fluorescent protein fusion construct (fig. S1). Like other genes in the aconitase metabolon, expression of *ACO1* becomes progressively HAP-dependent in response to increased respiratory activity when cells are shifted from glucose to raffinose medium, whereas a combined inactivation of the HAP and RTG systems leads to a 14-fold reduction of *ACO1* expression, independent of the carbon source (fig. S2).

To ask whether the HAP and RTG transcription complexes can directly affect mtDNA maintenance, we generated a diploid strain heterozygous for *hap2*Δ and *rtg1*Δ. After sporulation and dissection on rich

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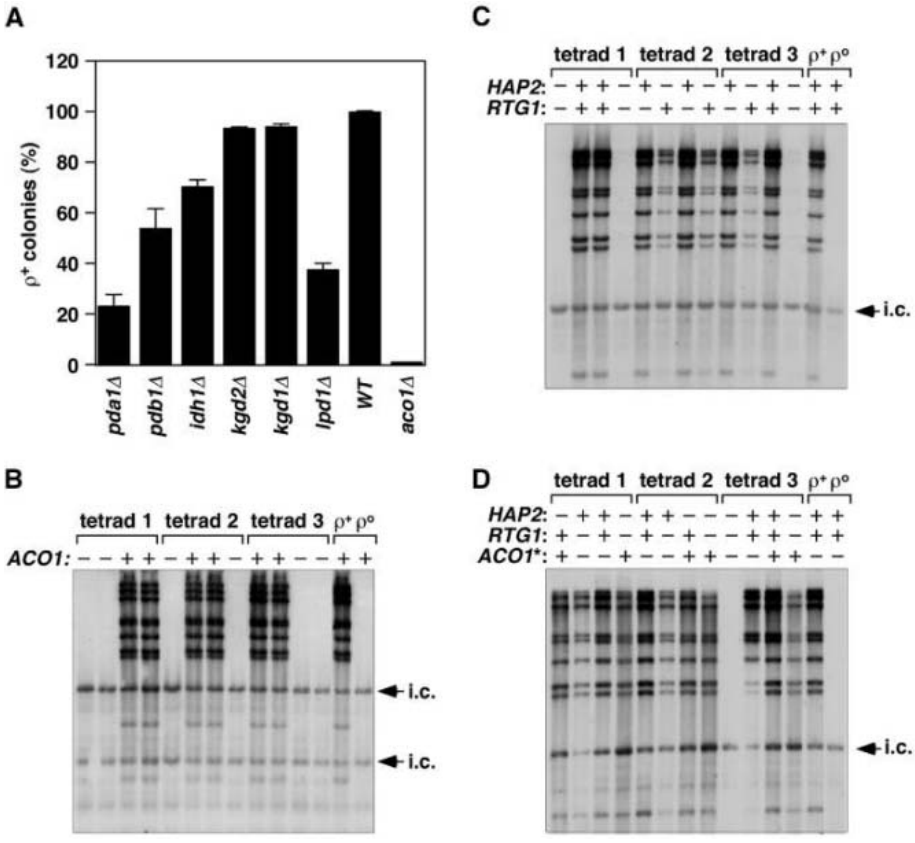

Fig. 1. *ACO1* is required for mtDNA maintenance. (**A**) mtDNA stability in cells with null mutations of genes that encode metabolic proteins found in nucleoids. mtDNA stability is expressed as percentage of respiratory-competent (ρ^+) colonies after growth for 35 generations in complete raffinose medium. Data are shown as means ± SEM for triplicate experiments. WT, wild type. (**B**) Southern blot analysis of *Cfo* I–digested total DNA probed for mtDNA in nascent meiotic segregants containing *ACO1* (+) or *aco1*Δ (–) alleles. Controls are ρ^+ and ρ^0 cells. i.c., internal control for sample loading. (**C**) Southern blot analysis showing the loss of mtDNA in *hap2*Δ *rtg1*Δ meiotic segregants. (**D**) Suppression of mtDNA loss from the *hap2*Δ *rtg1*Δ double mutants by constitutive expression of a chromosomally integrated copy of *ADH1-ACO1* (*ACO1**).

glucose medium, both the *rtg1*Δ and *hap2*Δ spores can maintain mtDNA (Fig. 1C), whereas mtDNA was barely detectable in *rtg1*Δ *hap2*Δ segregants. However, when the diploids contained an integrated single copy of *ACO1* under the control of the constitutive *ADH1* promoter, the loss of mtDNA from the *rtg1*Δ *hap2*Δ double mutants was largely reversed in meiotic segregants that also received the *ADH1-ACO1* allele (Fig. 1D).

To determine whether the mtDNA instability in *aco1*Δ cells is due to a block in metabolic flux through the aconitase metabolon, we inactivated all three known genes encoding CS: *CIT1*, *CIT2*, and *CIT3* encoding, respectively, the citric acid cycle CS, a peroxisomal isoform of CS, and a mitochondrial CS that may function in a methyl citrate pathway (*14*, *15*), so that no substrate would be available to aconitase. This resulted in a strong glutamate auxotrophy (Fig. 2A), demonstrating that metabolic flux through the aconitase metabolon had been blocked. Nevertheless, mtDNA was stable in the *cit1*Δ, *cit2*Δ, and *cit3*Δ triple mutant (Fig. 2B).

We next asked whether aconitase catalytic activity is required for Aco1p function in mtDNA maintenance. Aconitase contains an iron-sulfur center (4Fe-4S) in which 3 of the 4 mol of iron are coordinated with three cysteines, Cys^{382}, Cys^{445}, and Cys^{448} (*16*); the fourth iron is coordinated with the substrate, citrate (*17*). The integrity of this iron sulfur center is essential for aconitase enzymatic activity (*18*). We changed each of these cysteine residues to a serine and analyzed the mutants to see whether they could still function in mtDNA maintenance. Each mutant allele tagged with *URA3* was inserted

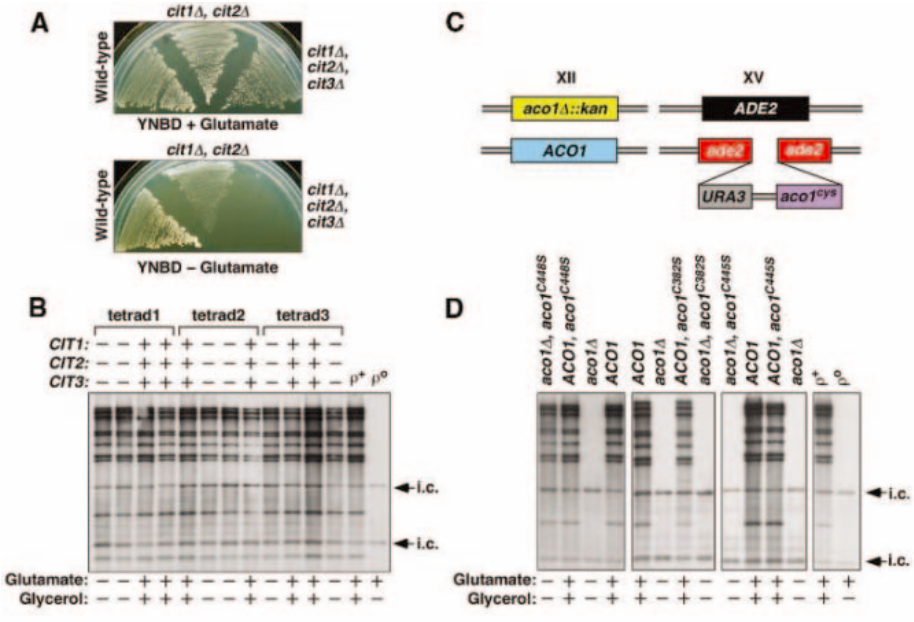

Fig. 2. Functional bisection of Aco1p. (**A**) Growth of wild-type and mutant cells with the indicated *cit*Δ alleles on minimal glucose medium with or without 0.2% glutamate (YNBD, 0.67% yeast nitrogen base, 2% dextrose). (**B**) Southern blot analysis of mtDNA in meiotic segregants from *CIT/cit*Δ diploid cells. (**C**) Schematic for generation of diploid strains used for the functional test of the cysteine mutants of *ACO1*. Chromosome locations are indicated (XII and XV). (**D**) Southern analysis showing that the *aco1*^{C448S} but not the *aco1*^{C382S} and *aco1*^{C445S} alleles retain mtDNA among the meiotic segregants in a representative tetrad.

into the *ADE2* locus of an *ACO1/aco1Δ::KAN* diploid strain (Fig. 2C). After sporulation, Southern blot analysis of meiotic segregants from a representative tetrad from each diploid strain showed that mtDNA was retained in *aco1Δ aco1*^{C448S} but not in the *aco1Δ aco1*^{C382S} or *aco1Δ aco1*^{C445S} segregants (Fig. 2D); however, all three of the segregants are glutamate auxotrophs and respiratory deficient, indicating that they lack aconitase activity. These differences in the ability of the *aco1*^{cys} mutant alleles to maintain mtDNA are not due to any appreciable differences in the expression of the mutant proteins, because all are expressed at levels comparable to wild-type Aco1p (fig. S3). Further experiments showed, however, that the *aco1*^{C382S} and *aco1*^{C445S} mutant alleles can support mtDNA maintenance when overexpressed from the strong *ADH1* promoter, although those mutants remain respiratory-deficient and auxotrophic for glutamate (fig. S4).

The requirement of Aco1p for mtDNA maintenance is comparable to that of the mtDNA packaging protein Abf2p, which is essential for mtDNA maintenance in cells grown on glucose medium (*6*). The only conditions known in which mtDNA in *abf2Δ* cells is relatively stable is when another DNA packaging protein, such as *Escherichia coli* HU, is expressed in *abf2Δ* cells and targeted to mitochondria (*19*) or when *abf2Δ* cells are grown on a nonfermentable carbon source (*6*). This latter result has generally been interpreted to mean that *abf2Δ* cells can be propagated on medium such as glycerol because of the strong selection for cells that retain mtDNA. However, under these growth conditions, *ACO1* expression is elevated (*10*). This raises the possibility that, under derepressed conditions, Aco1p might be able to substitute for Abf2p in mtDNA maintenance. To test this, we first examined mtDNA stability in *abf2Δ* cells grown on raffinose, which does not repress the aconitase metabolon. These experiments (Fig. 3A) show that growth on raffinose almost completely suppresses the mtDNA instability seen when *abf2Δ* cells are grown on glucose. Southern blot analysis of *abf2Δ* cells grown on glucose versus raffinose medium showed that after growth of the *abf2Δ* mutant in glucose for six generations, mtDNA was barely detectable in the cell population, whereas cells grown in raffinose for the same number of generations retained a much greater amount of mtDNA (Fig. 3B).

We next determined whether the HAP-RTG targets are responsible for the marked mtDNA stability observed in *abf2Δ* cells grown under derepressed conditions. We first manipulated the HAP2-5 transcription complex by constitutively expressing *HAP4*, which activates the expression of respiratory genes, including *ACO1*, in glucose-repressed cells (*20*). Accordingly, when *HAP4* was expressed from the constitutive *PGK1* promoter, the loss of mtDNA from *abf2Δ* cells grown on glucose was considerably delayed (Fig. 3C).

Next, we inactivated the *MKS1* gene encoding a negative regulator of the RTG pathway (*21*–*23*); this results in constitutive, high levels of expression of *RTG* target genes, including Aco1p. The *mks1Δ* mutation almost completely suppressed the loss of mtDNA from *abf2Δ* cells grown on glucose (Fig. 3D). That the *mks1Δ* suppressor activity acts through the RTG pathway is shown by the reappearance of mtDNA instability in the *abf2Δ mks1Δ* double mutant when *RTG1* or *RTG3* was also inactivated.

To show directly that the suppression of mtDNA instability of *abf2Δ* cells grown on glucose is related to *ACO1* expression, we expressed either the wild-type or one of the three cysteine mutants of Aco1p under control of the constitutive *ADH1* promoter in centromeric plasmids. The transformants, maintained on glycerol medium, were examined for mtDNA stability after plating on glucose medium. As expected, most *abf2Δ* cells transformed with vector alone formed petite colonies, indicating instability of mtDNA (Fig. 3E). However, in cells expressing wild-type Aco1p or its cysteine mutants, the mtDNA instability phenotype was reversed, yielding petite frequencies comparable to that of the control cells expressing *ABF2*.

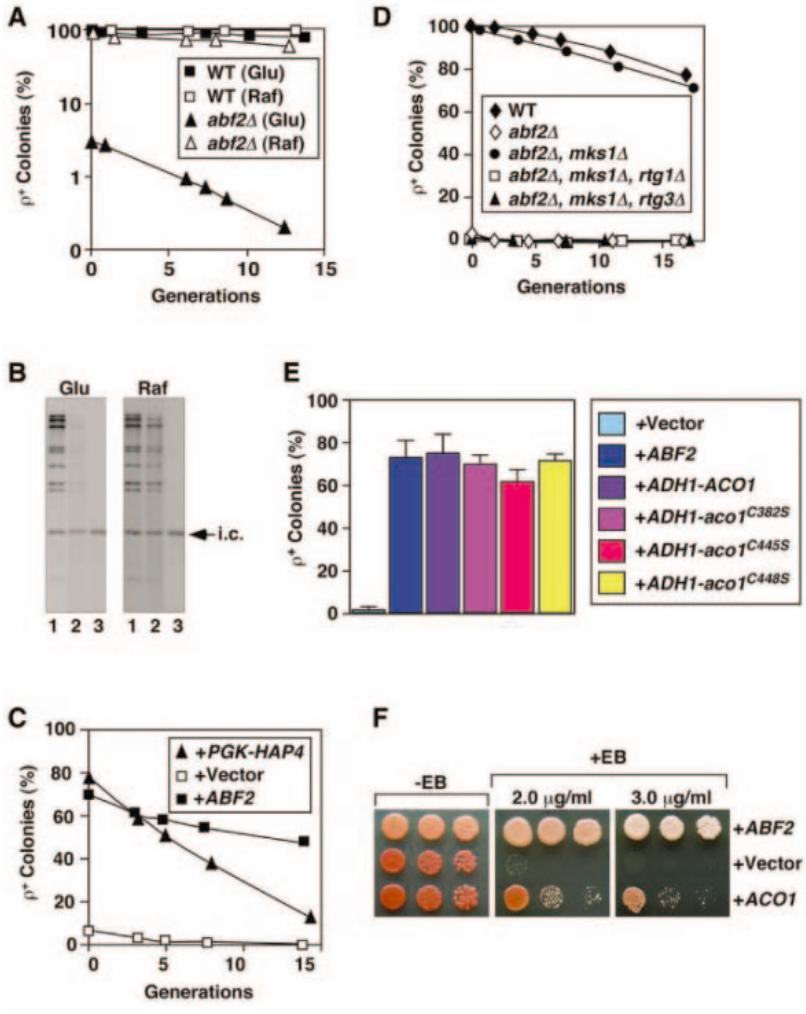

Fig. 3. Control of mtDNA inheritance by the HAP and RTG pathways. (**A**) mtDNA stability of *abf2Δ* cells grown in raffinose (Raf) or glucose (Glu) medium expressed as the percentage of ρ^+ colonies on complete glucose medium after a growth in liquid medium for the indicated generations. (**B**) Southern blot analysis of mtDNA in *abf2Δ* cells grown for six generations in complete glucose or raffinose medium. Lane 1, ρ^+ control; lane 2, *abf2Δ*; lane 3, ρ^o control; i.c., internal control. (**C**) Suppression of mtDNA instability in *abf2Δ* cells by overexpression of *HAP4* from the *PGK1* promoter in rich glucose medium. *abf2Δ* cells were also transformed with an empty vector and with wild-type *ABF2* on a plasmid as controls. (**D**) Suppression of mtDNA instability in *abf2Δ* cells by constitutive activation of the RTG pathway because of the *mks1Δ* mutation. (**E**) Suppression of mtDNA loss from *abf2Δ* cells by overexpression of *ACO1* and its C382S, C445S, and C448S variants from the constitutive *ADH1* promoter. mtDNA stability was determined by counting the fraction of ρ^+ colonies after plating transformants on complete glucose medium. (**F**) EB sensitivity of *abf2Δ* cells with *ABF2*, *ACO1*, or an empty vector on complete ethanol medium.

One interpretation for the suppression of mtDNA instability in *abf2*Δ cells by activation of *ACO1* is that mtDNA is repackaged into more stable nucleoid structures. To investigate this, we measured the sensitivity of ρ^+ cells to ethidium bromide (EB), which, by intercalating between the base pairs of mtDNA, produces petites because of the loss of the ρ^+ mtDNA. EB accessibility to DNA has been shown to be a useful probe for detecting differences in the organization of DNA-protein complexes (*24*). Indeed, by comparison to wild-type ρ^+ cells, *abf2*Δ cells are hypersensitive to EB as determined by growth on ethanol medium (Fig. 3F). This EB hypersensitivity can be suppressed by overexpression of *ACO1*. We confirmed that, under these growth conditions, expression of *ACO1* from the multicopy plasmid increases the steady-state level of Aco1p by threefold compared with that in cells containing the empty vector (fig. S5). Aco1p suppression of EB sensitivity of *abf2*Δ cells was also observed under glucose repressed conditions (fig. S6). Together, these data suggest that Aco1p may be providing some packaging function for mtDNA.

Our data establish a direct link between control of *ACO1* expression by the HAP and RTG pathways and the maintenance of mtDNA. Our finding that Aco1p is required for mtDNA stability independent of aconitase catalytic activity is reminiscent of the bifunctionality of the cytosolic form of mammalian aconitase, also known as iron-responsive element binding protein. That protein switches between an enzymatic and RNA binding form on the basis of the assembly or disassembly of the [4Fe-4S] cluster (*25*, *26*). Whether a similar mechanism applies to Aco1p function in mtDNA maintenance remains to be determined. Our results further suggest that mtDNA nucleoids may exist in different states depending on the metabolic condition of cells. Under glucose repressed conditions, Abf2p is essential for mtDNA maintenance because of its DNA packaging function. In derepressed cells with robust oxidative metabolism, or in response to RTG signals, mtDNA packaging may also involve Aco1p. This raises the possibility that nucleoid remodeling may be part of a strategy for adjusting mtDNA maintenance to the changes in cellular metabolism. Because aconitase is susceptible to oxidative damage during oxidative stress and cell aging (*27–29*), this property could contribute to compromised mtDNA integrity as a result of a decoupling between metabolism and mtDNA transactions.

Table 1. Mitochondrial nucleoid proteins identified by in organello formaldehyde cross-linking (supporting online text). ATP, adenosine triphosphate KGDC, α-ketoglutarate dehydrogenase complex; PHDC, pyruvate dehydrogenase complex; BCADC, branched-chain amino acids dehydrogenase complex. Genes encoding proteins identified in this study are boldfaced.

Gene	Protein	Primary functions
	Category I	
ABF2	Abf2p	mtDNA packaging, ρ^+ genome maintenance
MGM101	Mgm101p	Maintenance of ρ^+ and *ori*-less mtDNA, mtDNA repair
RIM1	Single-stranded DNA binding protein	mtDNA replication
RPO41	DNA-directed RNA polymerase	Mitochondrial transcription, ρ^+ mtDNA maintenance
SLS1	Sls1p	Mitochondrial translation, ρ^+ mtDNA maintenance
	Category II	
HSP60	mtHsp60p	Mitochondrial chaperonin
HSP10	mtHsp10p	Mitochondrial chaperonin
SSC1	mtHsp70p	Protein import
ATP1	α-Subunit of F_1-ATPase	ATP synthesis, protein import
	Category III	
ACO1	Mitochondrial aconitase	Citric acid cycle
ALD4	Aldehyde dehydrogenase	Ethanol metabolism
IDH1	NAD^+-dependent isocitrate dehydrogenase, subunit 1	Citric acid cycle
IDP1	$NADP^+$-dependent isocitrate dehydrogenase	Oxidative decarboxylation of isocitrate
KGD1	2-oxoglutarate dehydrogenase, E1 component of KGDC	Citric acid cycle
KGD2	2-oxoglutarate dehydrogenase, E2 component of KGDC	Citric acid cycle
LPD1	Dihydrolipoamide dehydrogenase, E3 component of PDHC, KGDC, and BCADC	Citric acid cycle, catabolism of branched-chain amino acids
LSC1	Succinate-CoA ligase, α subunit	Citric acid cycle
PDA1	Pyruvate dehydrogenase, E1 α-subunit of PDHC	Oxidation of pyruvate
PDB1	Pyruvate dehydrogenase, E1 β-subunit of PDHC	Oxidation of pyruvate
	Category IV	
ILV5	Acetohydroxyacid reductoisomerase	Biosynthesis of Val, Ile, and Leu
ILV6	Acetolactate synthase regulatory subunit	Biosynthesis of Val, Ile, and Leu
CHA1	L-serine/L-threonine deaminase	Catabolism of hydroxy amino acids

References and Notes

1. B. A. Kaufman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7772 (2000).
2. S. Meeusen *et al.*, *J. Cell Biol.* **145**, 291 (1999).
3. I. Miyakawa, N. Sando, K. Kawano, S. Nakamura, T. Kuroiwa, *J. Cell Sci.* **88**, 431 (1987).
4. N. Garrido *et al.*, *Mol. Biol. Cell* **14**, 1583 (2003).
5. D. F. Bogenhagen, Y. Wang, E. L. Shen, R. Kobayashi, *Mol. Cell. Proteomics* **2**, 1205 (2003).
6. J. F. Diffley, B. Stillman, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7864 (1991).
7. N. G. Larsson *et al.*, *Nature Genet.* **18**, 231 (1998).
8. B. A. Kaufman, J. E. Kolesar, P. S. Perlman, R. A. Butow, *J. Cell Biol.* **163**, 457 (2003).
9. H. Sato, A. Tachifuji, M. Tamura, I. Miyakawa, *Protoplasma* **219**, 51 (2002).
10. J. L. DeRisi, V. R. Iyer, P. O. Brown, *Science* **278**, 680 (1997).
11. M. T. McCammon, C. B. Epstein, B. Przybyla-Zawislak, L. McAlister-Henn, R. A. Butow, *Mol. Biol. Cell* **14**, 958 (2003).
12. Z. Liu, R. A. Butow, *Mol. Cell. Biol.* **19**, 6720 (1999).
13. R. A. Butow, N. G. Avadhani, *Mol. Cell* **14**, 1 (2004).
14. Y. K. Jia, A. M. Becam, C. J. Herbert, *Mol. Microbiol.* **24**, 53 (1997).
15. C. B. Epstein *et al.*, *Mol. Biol. Cell* **12**, 297 (2001).
16. A. H. Robbins, C. D. Stout, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3639 (1989).
17. H. Lauble, M. C. Kennedy, H. Beinert, C. D. Stout, *J. Mol. Biol.* **237**, 437 (1994).
18. H. Hirling, B. R. Henderson, L. C. Kuhn, *EMBO J.* **13**, 453 (1994).
19. T. L. Megraw, C. B. Chae, *J. Biol. Chem.* **268**, 12758 (1993).
20. J. Blom, M. J. De Mattos, L. A. Grivell, *Appl. Environ. Microbiol.* **66**, 1970 (2000).
21. I. Dilova, C.-Y. Chen, T. Powers, *Curr. Biol.* **12**, 389 (2002).
22. T. Sekito, Z. Liu, J. Thornton, R. A. Butow, *Mol. Biol. Cell* **13**, 795 (2002).
23. J. J. Tate, K. H. Cox, R. Rai, T. G. Cooper, *J. Biol. Chem.* **277**, 20477 (2002).
24. J.-J. Lawrence, M. Daune, *Biochemistry* **15**, 3301 (1976).
25. M. C. Kennedy, L. Mende-Mueller, G. A. Blondin, H. Beinert, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 11730 (1992).
26. R. D. Klausner, T. A. Rouault, *Mol. Biol. Cell* **4**, 1 (1993).
27. M. D. Williams *et al.*, *J. Biol. Chem.* **273**, 28510 (1998).
28. L. J. Yan, R. L. Levine, R. S. Sohal, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 11168 (1997).
29. J. L. Bradley *et al.*, *Hum. Mol. Genet.* **9**, 275 (2000).

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Natural Selection and Developmental Constraints in the Evolution of Allometries

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In animals, scaling relationships between appendages and body size exhibit high interspecific variation but low intraspecific variation. This pattern could result from natural selection for specific allometries or from developmental constraints on patterns of differential growth. We performed artificial selection on the allometry between forewing area and body size in a butterfly to test for developmental constraints, and then used the resultant increased range of phenotypic variation to quantify natural selection on the scaling relationship. Our results show that the short-term evolution of allometries is not limited by developmental constraints. Instead, scaling relationships are shaped by strong natural selection.

Among species, populations, and even sexes, morphological traits exhibit an impressive diversity of scaling relationships with body size; most traits scale positively with body size, although the rate at which trait size changes with overall size often differs from isometry and can even be nonlinear (*1*, *2*). This is particularly true of insects, which exhibit extremes in trait–body size allometries (*3*). This extreme variation among groups is in marked contrast to the extent of variation within groups; typically, individuals within these groups exhibit low variation around some average allometry, reflecting a tight scaling between body parts and overall size [e.g., (*4*–*7*)].

Although these patterns have long been recognized (*2*, *8*), surprisingly little is known about the evolution of scaling relationships (*3*, *9*); in particular, the relative importance of processes shaping their evolution is largely uninvestigated (*10*). Presumably, tight adherence to particular allometries results from external selection against traits with atypical or nonfunctional relative sizes. Such selection is predicted to favor the evolution of genetic and developmental systems that properly scale the growth of traits across body sizes, maintaining functional size relationships in the face of environmental and genetic variation (*11*, *12*). However, this scenario presents a paradox: The proximate mechanisms that evolve to maintain the relative size of traits will then produce developmental constraints [as defined in (*10*)] that must be overcome if allometries are to evolve. Here, we present empirical data addressing the relative roles of natural selection and developmental constraints in the evolution of the allometry between forewing area and body size in the butterfly *Bicyclus anynana*.

In the context of the evolution of allometries among morphological traits, the scaling relationship between wing area and body size (i.e., the ratio of body size to wing area, or "wing loading") is of interest, because it is ecologically important and taxonomically diverse (*13*, *14*). The size of the wings and flight musculature relative to body mass affects flight performance directly (*14*), as well as indirectly through thermoregulatory effects while basking or during ectotherm flight (*13*). Lepidoptera have the lowest average wing loading among flying insects (*15*) and exhibit lineage-specific, seasonal morph–specific, or sex-specific scaling relationships associated with life historical or behavioral correlates [e.g., (*6*, *16*–*20*)]. As with most insects, adult body size in *B. anynana* is a highly plastic trait (*21*), and forewing area (FW) exhibits a strong, positive phenotypic correlation with total body mass (BS) across the natural range of body size (Pearson correlation coefficient = 0.86, $N = 691$ stock population females, $P = 0.0001$). Moreover, artificial selection for changes in FW and pupal mass revealed a genetic correlation between these traits ($r = 0.75$) (Fig. 1) (*20*).

The strong genetic correlation between FW and body size should constrain their independent evolution (*22*), inhibiting phenotype evolution in a direction perpendicular to that of the wild-type allometry (*23*). To determine whether such internal constraints limit the short-term evolution of the scaling relationship, we performed artificial selection on the FW/BS allometry (*20*). The FW/BS allometry evolved rapidly, diverging ~2 SD in each direction relative to that of the control lineage to produce distinct, novel phenotypes [Fig. 2; discriminant function analysis correctly classified 94.8% of females from generation 13 (–2 log likelihood = 107.4; $N = 766$, replicates pooled)]. The response to selection resulted almost entirely from changes in FW (Fig. 3); BS changed in the appropriate direction in only one lineage (–FW/+BS, lineage E; $F_{1,11} = 5.55$, $P = 0.038$) (*20*). This extreme asymmetry in the contribution of each trait to the evolution of the allometry was unexpected, as both individual FW and body size exhibited very similar realized heritabilities (Fig. 1), indicating adequate and equivalent genetic variation in both traits. Moreover, the observed pattern of response is not due to differences in the phenotypic variance between the traits, because they were subject to similar indirect selection pressures in all but one case (Fig. 3). A low frequency of alleles in our starting population that affect BS independently of FW or a sieving out of key alleles that affect FW but not BS could account for the pattern. In any case, the rapid evolution of the allometry demonstrates a surprising absence of developmental constraints restricting change in this scaling relationship. However, the pattern of response exhibited by FW and BS indicates a strong

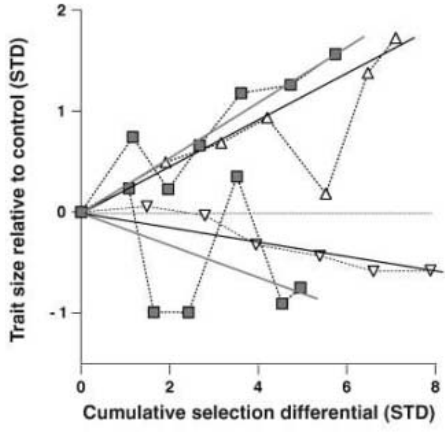

Fig. 1. Response to artificial selection on absolute trait size. Forewing area (FW, triangles indicate direction of selection) and pupal mass (PM, squares) are shown relative to control means (horizontal line) as a function of the cumulated selected differential. Regression was used to calculate the realized heritability for each trait. Pupal mass increased ~1.5 SD and decreased ~1 SD in six generations. Realized heritabilities were moderate in each direction (+PM $h^2 = 0.28$, –PM $h^2 = 0.16$). FW responded rapidly and asymmetrically to selection, increasing ~1.5 SD and decreasing ~0.5 SD relative to controls. Realized heritabilities were moderate and similar to those for pupal mass [+FW $h^2 = 0.38$, –FW $h^2 = 0.16$; note that these values are twice the slope of the regression for FW because only females were selected in these lineages (*20*)].

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bias [or developmental constraint (*10*)] in how these traits respond indirectly to direct selection on their scaling relationship. Hence, the allometry itself is not developmentally constrained; what does appear to be constrained is the way in which the individual components contribute to the evolution of this complex phenotype.

Our results, together with the few other studies that have used artificial selection to alter scaling relationships between morphological traits in insects (*24*–*26*), indicate that even strong genetic correlations do not constrain phenotype evolution in the short term. It seems that the developmental basis of these genetic correlations is more important than their strength in determining the response to selection (*27*). In particular, under novel selection regimes such as the artificial one we imposed, the developmental program coordinating the growth of the individual traits may influence how these traits and the relationship between them evolves (*28*).

The lack of developmental constraints on the evolution of the allometry motivated us to determine the pattern of natural selection on wing loading. To examine the fitness consequences of deviating from the wild-type FW/BS scaling relationship, we measured the mating success of competing wild-type control and novel-phenotype males (two treatment male classes, +FW/–BS and –FW/+BS) in a spacious, naturally planted, tropical greenhouse. Treatment and control males taken from reciprocal crosses of the replicated lineages of each selected direction were selected for inclusion in the experiment on the basis of their static allometries (*20*). Hence, all males came from similarly outcrossed populations, and treatment and control males were drawn from the same genetic background. Mating success was determined by the transfer of phenotype class–specific colored powder from males to females (*20*, *29*). In both trials, males with the wild-type phenotype acquired three times as many matings as did males from both phenotype classes with novel wing loadings (Fig. 4; trial 1, $G = 30.2$, $P < 0.001$; trial 2, $G = 18.381$, $P < 0.001$). These results demonstrate strong stabilizing selection favoring the natural scaling relationship between forewing and body size in *B. anynana*.

Survival among male phenotype classes (recapture rates) did not differ (trial 1, $G = 0.110$, $P = 0.947$; trial 2, $G = 0.641$, $P = 0.726$), a finding consistent with results from manipulative studies of wing loading in free-flying butterflies [e.g., (*15*, *30*, *31*)]. Because survival was the same among male phenotypes, the higher fitness of wild-type males must be due to other, nonexclusive, selective factors. In the greenhouse, males engage in prolonged bouts of chasing both other males and females, as they do in nature (*29*), which suggests that the lower fitness of treatment males may result from decreased locomotor performance (*20*). Intrasexual competition among male phenotypes may also play a role; +FW/–BS and –FW/+BS males may be excluded from females in the presence of superiorly flying, wild-type males. Alternatively, wild-type males may be selected by females because of favored signals produced during courtship or flight (*29*) or because treatment males are less appealing visually. In any case, because treatment and wild-type males were drawn from the same outbred populations, any female preference must be largely unrelated to male genetic background in our experiment. Regardless of the cause of the higher fitness of wild-type males, we

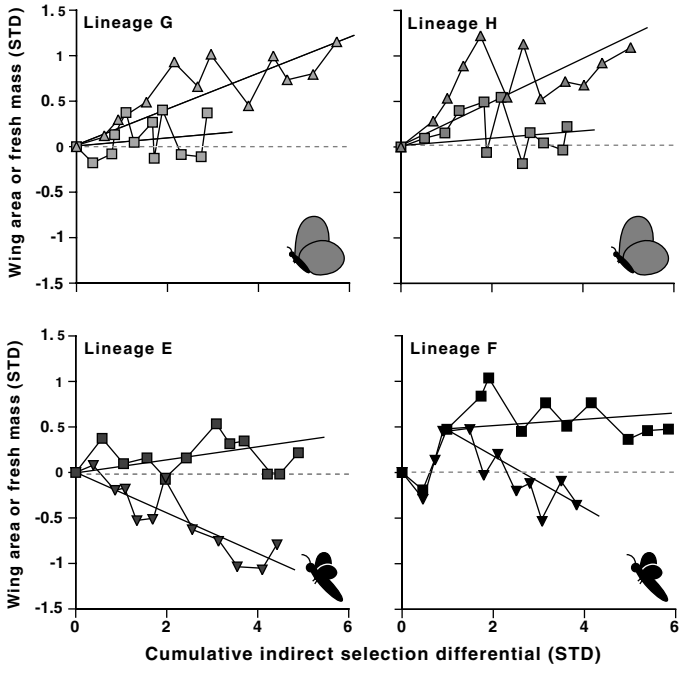

Fig. 3. Indirect response of mean forewing area (triangles indicate direction of FW selection relative to fresh mass) and fresh mass (squares) to direct selection on their scaling relationship. Values are plotted relative to control lineage means (horizontal dashed line) as a function of the cumulative indirect selection on each trait. The average indirect response of each trait is shown by the individual regression line for that trait. In lineage F, divergence in the values did not occur until generation 4; hence, the mean indirect response to selection is shown from generation 3 onward for this replicate. Cartoons represent the selected target phenotype.

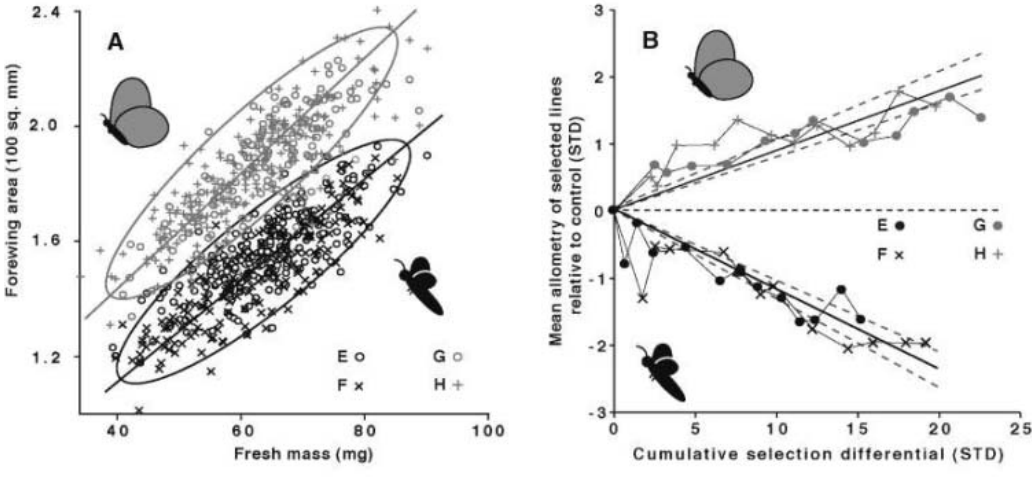

Fig. 2. Scatterplots of static allometries of individuals from lineages selected for changes in FW/BS and the evolution of the mean allometry. (**A**) Phenotype distributions of lineages selected for changes in forewing–body size scaling. Each selected population is shown as a different symbol; replicates of a selection direction have the same shading. The mean allometry of each selected direction (replicates combined) is shown as the model II regression through the points and is enclosed by a 95% confidence ellipse. (**B**) Realized heritabilities of the mean allometries. Mean phenotype (through which the mean allometry passes) for each lineage is shown relative to control values (horizontal dashed line) as a function of the cumulative selection differential. Mean heritabilities (+FW/–HW = 0.18; –FW/+HW = 0.24) are equal to twice the slope of the regressions fit to each selected direction (95% confidence intervals shown by dashed lines). Target phenotypes are represented by cartoons in both panels.

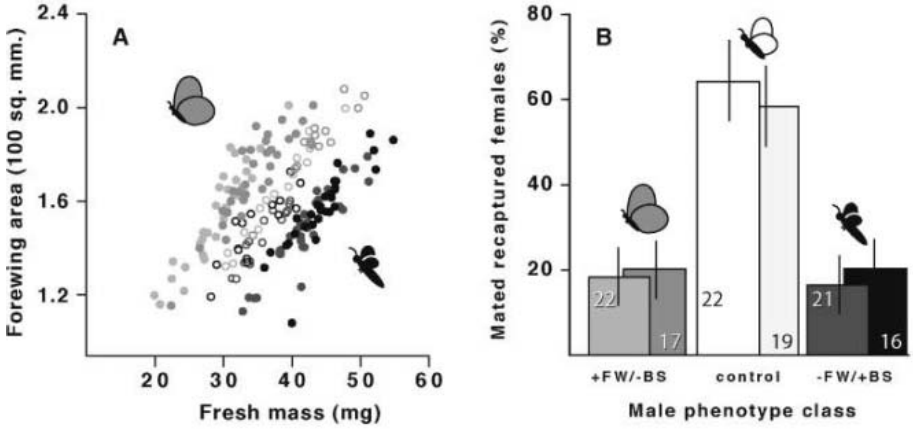

Fig. 4. Distributions of static allometries for FW/BS allometries and relative mating success of three male phenotype classes. (**A**) Distributions of individual males with +FW/–BM, wild type, and –FW/+BM phenotypes included in the experiment (extreme phenotypes are represented by cartoons). Solid circles denote novel phenotype classes; open circles denote wild-type controls. (**B**) Mating success of each phenotype class. Columns indicate percentage of recaptured females that mated with males in each class and are shown with 95% confidence intervals based on a bimodal distribution. Numbers in the columns indicate the number of males recaptured in each group. Data from replicate trials are indicated by similar shading (shared between panels).

have documented strong stabilizing selection on male wing loading.

Our findings indicate that it is not internal developmental constraints, but rather external natural selection, that is the primary force shaping the short-term evolution of morphological allometries in insects. However, the surprising bias in the morphological basis of how the allometry evolved suggests that development may strongly influence how individual traits respond to selection on their scaling relationships.

References and Notes

1. D. W. Thompson, *On Growth and Form* (Cambridge Univ. Press, Cambridge, 1917).
2. J. S. Huxley, *Problems of Relative Growth* (Methuen, London, 1932).
3. D. J. Emlen, H. F. Nijhout, *Annu. Rev. Entomol.* **45**, 661 (2000).
4. D. Burkhardt, I. de la Motte, *Entomol. Gen.* **12**, 221 (1987).
5. W. G. Eberhard, E. E. Gutierrez, *Evolution* **45**, 18 (1991).
6. R. E. Strauss, in *Ordination in the Study of Morphology, Evolution, and Systematics of Insects: Applications and Quantitative Genetic Rationales*, J. T. Sorensen, R. Foottit, Eds. (Elsevier Science, Amsterdam, 1992), pp. 157–179.
7. D. J. Emlen, *Science* **291**, 1534 (2001).
8. S. J. Gould, *Biol. Rev.* **41**, 587 (1966).
9. D. L. Stern, D. J. Emlen, *Development* **126**, 1091 (1999).
10. J. Maynard Smith *et al.*, *Q. Rev. Biol.* **60**, 265 (1985).
11. G. P. Wagner, *Am. Zool.* **36**, 36 (1996).
12. G. P. Wagner, G. Booth, H. Bagheri-Chaichian, *Evolution* **51**, 329 (1997).
13. R. Dudley, *The Biomechanics of Insect Flight: Form, Function, Evolution* (Princeton Univ. Press, Princeton, NJ, 2000).
14. S. Vogel, *Comparative Biomechanics* (Princeton Univ. Press, Princeton, NJ, 2003).
15. J. G. Kingsolver, *Evolution* **53**, 1479 (1999).
16. For example, low-flight butterflies such as non-dispersers or perching, sit-and-wait males have lower ratios of flight muscle mass to wing size than do stronger flying butterflies such as dispersing individuals or males that actively patrol for females [reviewed in (*19*)].
17. R. E. Strauss, *Evolution* **44**, 86 (1990).
18. P. Wickman, *Evolution* **46**, 1525 (1992).
19. H. Van Dyck, E. Matthysen, *Trends Ecol. Evol.* **14**, 172 (1999).
20. See supporting data on *Science* Online.
21. R. E. Kooi, P. M. Brakefield, *Entomol. Exp. Appl.* **80**, 149 (1996).
22. D. S. Falconer, T. F. C. MacKay, *Introduction to Quantitative Genetics* (Addison-Wesley Longman, Essex, UK, ed. 4, 1997).
23. D. Schluter, *Evolution* **50**, 1766 (1996).
24. K. E. Weber, *Genetics* **126**, 975 (1990).
25. G. S. Wilkinson, *Genet. Res.* **62**, 213 (1993).
26. D. J. Emlen, *Evolution* **50**, 1219 (1996).
27. J. B. Wolf, W. A. Frankino, A. F. Agrawal, E. D. Brodie III, A. J. Moore, *Evolution* **55**, 232 (2001).
28. S. H. Rice, *Evolution* **52**, 647 (1998).
29. M. Joron, P. M. Brakefield, *Nature* **424**, 191 (2003).
30. R. B. Srygley, J. G. Kingsolver, *Biol. J. Linn. Soc.* **70**, 707 (2000).
31. J. G. Kingsolver, R. B. Srygley, *Evol. Ecol. Res.* **2**, 593 (2000).
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References

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Mechanisms of Hair Graying: Incomplete Melanocyte Stem Cell Maintenance in the Niche

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Hair graying is the most obvious sign of aging in humans, yet its mechanism is largely unknown. Here, we used melanocyte-tagged transgenic mice and aging human hair follicles to demonstrate that hair graying is caused by defective self-maintenance of melanocyte stem cells. This process is accelerated dramatically with *Bcl2* deficiency, which causes selective apoptosis of melanocyte stem cells, but not of differentiated melanocytes, within the niche at their entry into the dormant state. Furthermore, physiologic aging of melanocyte stem cells was associated with ectopic pigmentation or differentiation within the niche, a process accelerated by mutation of the melanocyte master transcriptional regulator *Mitf*.

Qualitative and quantitative changes in stem and progenitor cells have been implicated in physiological (chronological) aging (*1*, *2*), although the changes are poorly understood and the process of stem-cell aging has not been visually observed. Involvement of stem and progenitor cells in aging of multiple organ systems has been suggested in mice defective in DNA damage repair and telomere maintenance (*3*), but melanocytes may be unique in that the oxidative chemistry of melanin biosynthesis can be cytotoxic (*4*). This led to the suggestion that differentiated, pigmented melanocytes (rather than their unpigmented progenitors) are specifically targeted in hair graying (*5*, *6*). The recent discovery of unpigmented melanocyte stem cells, distinctly located within the hair follicle (*7*), creates an opportunity to determine whether the process of hair graying arises specifically from changes in differentiated melanocytes or the stem-cell pool that provides them.

Stem cells are maintained in the niche microenvironment (*8*). Hair follicles contain a well-demarcated structure for the stem-cell niche (within the lower permanent portion), whereas differentiated melanocytes reside in the hair bulb (at the base of the transient portion of the hair follicle) (Fig. 1A) (*7*, *9*).

Hair follicles are constantly renewing, with alternating phases of growth (anagen), regression (catagen), and rest (telogen) (fig. S1). Taking advantage of the spatial segregation of the stem versus differentiated cell compartments (*7*), we used melanocyte-targeted (Dct) lacZ transgenic mice (*7*, *10*, *11*) to examine the impact of aging on these melanocyte compartments.

Among hair graying models, the melanocyte lineage in *Bcl2*$^{-/-}$ (*12*) and *Mitf*$^{vit/vit}$ (*13*) mice show relatively selective hair graying compared with mouse models of syndromic premature aging, which affect numerous cell lineages (*14*, *15*). Hair graying in the *Bcl2*$^{-/-}$ background has been suggested to arise by chemical cytotoxity of melanin synthesis (*5*, *12*, *16*, *17*). Distribution and morphology of melanoblasts among *Bcl2*$^{-/-}$, *Bcl2*$^{-/+}$, and *Bcl2*$^{+/+}$ mice were normal during early development (fig. S2, a to h). *Bcl2*$^{-/-}$ mice gray after the first hair molting (Fig. 1, B and C) with white hairs. Histologically, differentiated melanocytes were almost completely absent in *Bcl2*$^{-/-}$ pelage (body hair) or whisker follicles (Fig. 1, E and G) compared with *Bcl2*$^{-/+}$ (Fig. 1, D, F, and H) or *Bcl2*$^{+/+}$ (*18*) follicles at postnatal day 39 (P39). Albino background did not protect against melanocyte loss in *Bcl2*$^{-/-}$ mice (Fig. 1I), suggesting that melanin synthesis is unnecessary for this melanocyte disappearance. In addition, *Bcl2*$^{-/-}$ follicles in the second hair cycle lack both differentiated melanocytes in the hair bulb and undifferentiated Dct-lacZ^{+} melanoblasts in the stem-cell niche (located at the bulge area in pelage follicles) (Fig. 1, D and E, and fig. S2, i and j), suggesting that *Bcl2* might be important for survival of melanocyte stem cells.

Looking earlier at P6.5, when hair follicle morphogenesis is almost complete, *Bcl2*$^{-/-}$ follicles appear normal (Fig. 2B). In contrast, *Bcl2*$^{-/-}$ follicles at P8.5 showed sudden, nearly complete loss of melanoblasts in the niche (bulge area, Fig. 2D), whereas the number of melanocytes in the hair bulb did not show significant differences between *Bcl2*$^{-/+}$ and *Bcl2*$^{-/-}$ mice (Fig. 2E). In both pelage and whisker follicles from *Bcl2*$^{-/-}$ animals, disappearance of niche melanoblasts begins at stage 6 of hair follicle morphogenesis [standardized hair follicle stages based on (*19*)], and by stage 8 they are gone (fig. S2, k to n). At this stage, niche melanoblasts undergo a morphologic change from a dendritic shape into a slender, oval shape with shrinkage to maximal nuclear/cytoplasmic ratio upon entry into the dormant state (Fig. 2, F and G). This change in morphology was seen cyclically at corresponding stages of subsequent cycles (*18*). Apoptosis of melanocyte stem cells was observed at the same stage on the albino or black background (*Tyr*$^{c\text{-}2j/c\text{-}2j}$) in both pelage and whisker hair follicles (Fig. 2, H to M). The same pattern of cell loss was detected by using Dct-lacZ, KIT (c-Kit) (KIT), or microphthalmia-associated transcription factor (MITF) as markers (fig. S3 and Fig. 3, A and B). On the other hand, melanocytes in the epidermis and dermis of hairless skin (e.g., tail and soles) survived throughout the hair regeneration cycle (fig. S2, o and p). These findings indicate that BCL2 selectively protects melanocyte stem cells at the time of their transition into the dormant state in the niche and could potentially be responsible for certain forms of human presenile hair graying, although no direct supporting evidence has been reported thus far.

In contrast to *Bcl2*$^{-/-}$, the *Mitf*$^{vit/vit}$ (*13*) graying mouse model exhibited a gradual decrease of melanocyte stem cells rather than abrupt loss (figs. S4 and S6). This strain contains a mild hypomorphic mutation in *Mitf*, the melanocyte master transcriptional regulator [(*20*, *21*) and references therein]. At early to mid-anagen of the third hair cycle, lacZ^{+} cells left in the niche of *Mitf*$^{vit/vit}$ pelage follicles and *Mitf*$^{vit/+}$ whisker follicles often produced melanin pigment and exhibited a bipolar or dendritic morphology (Fig. 3, C and D, and fig. S4, j and s). These pigmented cells are unusual because the niche of wild-type controls contains only unpigmented mel-

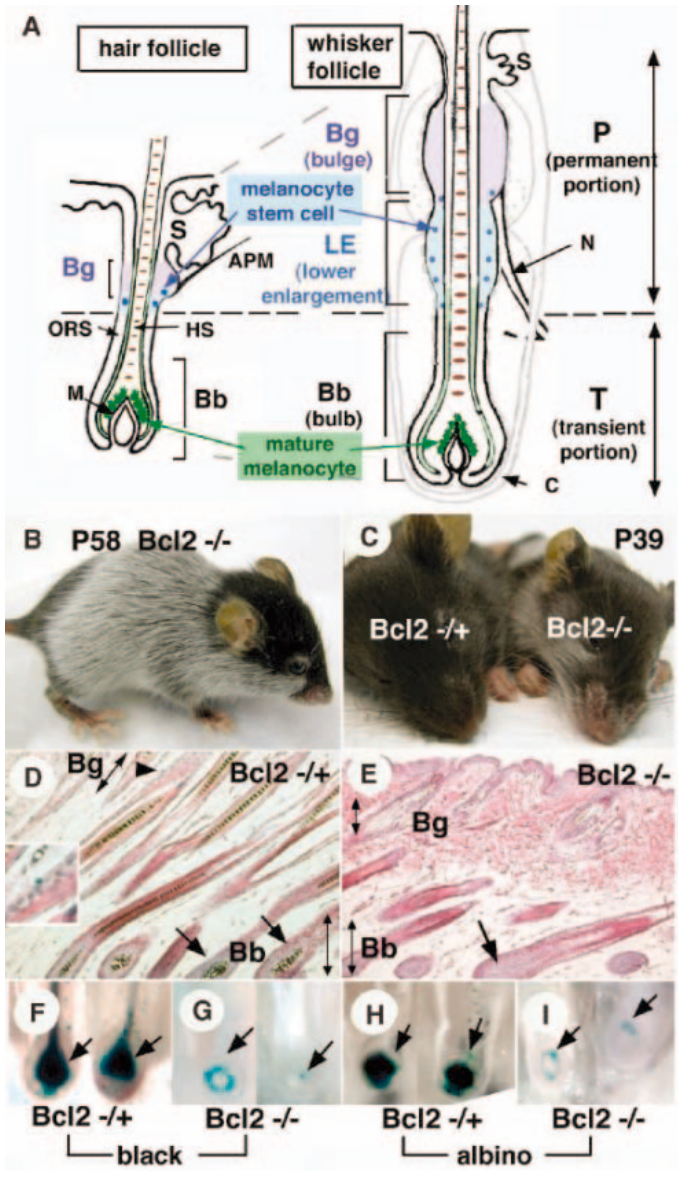

Fig. 1. Differentiated melanocytes are lost in the hair bulb of *Bcl-2* deficient mice. (**A**) Hair follicle structure. Melanocyte stem cells (blue dots) are in the lower permanent portion (light blue): the bulge (Bg) area in pelage follicles and the lower enlargement (LE) in whisker follicles. APM, arrector pili muscle (Figure 1A legend in SOM). (**B**) Appearance of *Bcl2*$^{-/-}$ mouse at P58. (**C**) Hair graying of whiskers in *Bcl2*$^{-/-}$ mouse at P39. (**D** and **E**) Distribution of lacZ^{+} cells (melanocytes) in P39 *Bcl2*$^{-/+}$ and *Bcl2*$^{-/-}$ mice carrying the *Dct-lacZ* transgene. Pigmented melanocytes in the bulb (Bb) [arrows in (D)] and lacZ^{+} melanoblasts in the Bg [arrowhead in (D); the inset shows the magnified view] are completely lost in *Bcl2*$^{-/-}$ follicles (E). Double arrows indicate the level of Bb or Bg. Magnification is 100×. (**F** to **I**) Whole-mount lacZ staining of the bulb of whisker follicles from *Bcl2*$^{-/+}$ and *Bcl2*$^{-/-}$ in black and white (albino: *Tyr*$^{c\text{-}2j/c\text{-}2j}$) backgrounds at P40. Loss of Dct-lacZ^{+} melanocytes was detected in the bulb of *Bcl2*$^{-/-}$ whisker follicles regardless of albino background.

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anocyte stem cells. We provisionally use the term ectopic pigmentation or differentiation for this reproducibly observed population because it is uncertain by which pathway these cells became pigmented, although they were absent in age-matched controls whose niche melanoblasts remain undifferentiated (Fig. 3E and fig. S4u).

Physiologic (senile) aging in mice also produces hair graying (fig S5), which could be caused by loss of melanocyte stem cells. Indeed during physiologic aging, niche melanoblasts ($lacZ^+$) were lost in a gradual and progressive fashion (Fig. 3, F and G). Moreover, whole-mount cross sections of 8-month-old follicles revealed pigment-containing melanocytes within the stem-cell niche in addition to their scattered distribution in the outer root sheath below the niche in whisker follicles (Fig. 3, H and I, and fig. S5, k to n). The appearance of these pigmented melanocytes in the niche is reminiscent of pigmented niche melanocytes observed during the accelerated graying of *Mitf-vit* mutants. Quantitative analysis revealed that the presence of these cells was accompanied by simultaneous loss of the typical unpigmented Dct-$lacZ^+$ melanoblasts in the niche and correlated closely with aging (Fig. 3, F and G). Thus, self-maintenance of melanocyte stem cells is essentially complete in young animals but becomes defective with aging.

We also analyzed the distribution of melanoblasts in aging human hair follicles with the use of MITF immunostaining (Fig. 4). $MITF^+$ small unpigmented melanoblasts were found in the outer root sheath preferentially around the bulge area where the arrector pili muscle attaches below the level of the sebaceous gland (Fig. 4, A to C), similar to previously described amelanotic melanocytes (*22*, *23*) that express PMEL17 (*24*, *25*), a transcriptional target of MITF (*26*). These cells have been suggested to be a reservoir population for differentiated melanocytes (*23*) and exhibit very similar morphology to melanocyte stem cells in mice. Whereas $MITF^+$ immature melanoblasts were abundant in follicles from 20- to 30-year-old subjects (2 to 3% of the total basal keratinocytes in the bulge area), they were absent from most hair follicles of 70- to 90-year-old subjects (Fig. 4J). $MITF^+$ melanocytes in the uppermost area (infundibulum) of the outer root sheath did not decrease significantly with aging, thus serving as a control population in these studies (fig. S7).

Follicles from intermediate-aged individuals (40 to 60 years old) revealed intermediate loss of bulge melanoblasts (Fig. 4, C and J). Bulge melanoblasts were found more in pigmented follicles than in gray follicles (*18*), as shown recently with $PMEL17^+$ bulge melanoblasts of middle-aged individuals (*27*). In addition, as with aged or $Mitf^{vit}$ mouse follicles, ectopically pigmented $MITF^+$ cells were occasionally observed in the bulge area or just below. These cells closely resembled the dendritic melanocytes described by Narisawa *et al*. in the bulge area of human follicles (*28*). The ectopically pigmented or differentitated melanocytes were seen exclusively in middle-aged follicles but did not accumulate in the bulge area, suggesting that they are not self-maintaining.

Our results demonstrate that *Bcl2* is selectively critical for maintenance of melanocyte stem cells, specifically for entry into the dormant state. *Bcl2* was previously shown to modulate hematopoietic stem-cell pool size (*29*). Different lineages might use distinct antiapoptotic mechanisms to resist the specific stress signals for dormancy.

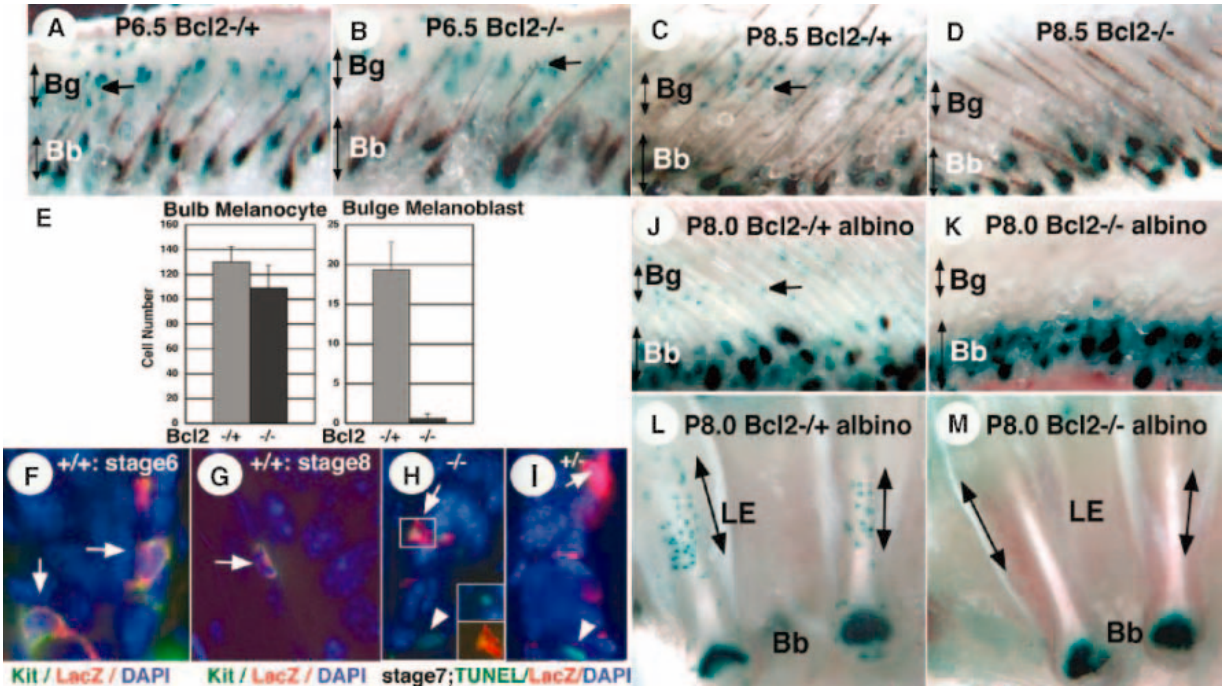

Fig. 2. Loss of $Bcl2^{-/-}$ melanocyte stem cells upon entry into the dormant state. (**A** to **D**) Distribution of Dct-$lacZ^+$ melanoblasts (arrows) in the Bg (top double arrow) of pelage follicles at P6.5 and P8.5. Whereas Bb melanocytes appear largely unchanged (bottom double arrow), bulge melanoblasts are lost in $Bcl2^{-/-}$ follicles at P8.5 [compare (D) with (C)] but not at P6.5 [compare (B) with (A)]. (**E**) Comparison of the total number per field of Dct-$lacZ^+$ melanoblasts in the bulb versus in the bulge plus subbulge of $Bcl2^{-/-}$ and $Bcl2^{-/+}$ pelage follicles at P8.5 on 7-μm sections (magnified 100×). KIT expression matches Dct-$lacZ^+$ in bulge melanoblasts [stage 6 (**F**) and stage 8 (**G**)] of $Bcl2^{+/+}$ animals (magnification, 630×). Cell size is diminished from stage 6 to stage 8. Terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling (TUNEL), lacZ, and 4′,6′-diamidino-2-phenylindole (DAPI) staining of stage 7 skin from P6.5 $Bcl2^{-/-}$ (**H**) and P6.5 $Bcl2^{+/-}$ (**I**) mice. Arrowheads show apoptotic inner root sheath keratinocytes. The inset area, marked with the arrow in (H), shows an apoptotic melanoblast. The top inset on the right shows the merged view for TUNEL (green) and DAPI (blue). The bottom inset shows the merged view for TUNEL (green) and LacZ (red). Distribution of Dct-$lacZ^+$ melanoblasts in the niche: Bg of pelage hair follicles (**J** and **K**) and LE (double arrow) of whisker hair follicles [(**L** and **M**), double arrows] from P8.0 mice with white backgrounds ($Tyr^{c\text{-}2j/c\text{-}2j}$).

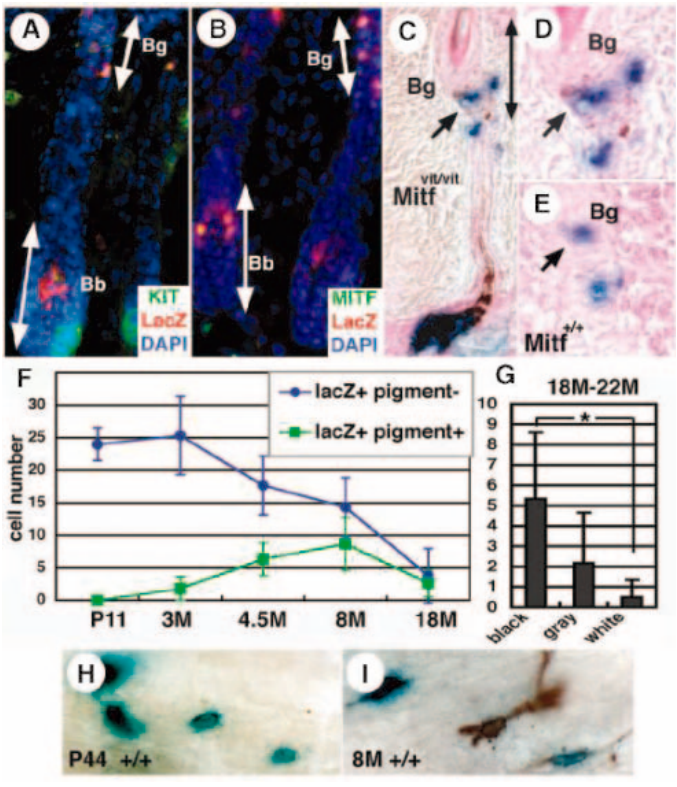

Fig. 3. Effect of aging and *Mitf* mutation on melanocyte stem cells. (**A** and **B**) Coincident expression of Dct-lacZ, KIT, and MITF in hair follicle melanoblasts or melanocytes (magnification, 200×). (See fig. S3 for more details.) (**C**) Ectopically pigmented melanoblasts (lacZ$^+$, blue) in the bulge region (arrow) of 3.5-month-old *Mitf*$^{vit/vit}$ follicles. (**D**) Magnified view of pigmented bulge melanoblasts. (**E**) Absence of pigment in lacZ$^+$ bulge melanoblasts of age-matched *Mitf*$^{+/+}$ follicles. (**F**) Quantitation of niche melanocytes (lacZ$^+$), either unpigmented (classical stem cells, blue) or ectopically pigmented (green), in LE of whisker follicles (positions a2 and a3) (see fig. S1i for positions). (**G**) Number of unpigmented niche melanoblasts in whisker follicles (positions a2, a3, c5, and d5) with black, gray, and white hair in 18- to 22-month-old (18–22M) wild-type mice. Asterisk indicates statistical significance ($P < 0.01$). (**H** and **I**) Ectopically pigmented melanoblasts in the niche (LE of whisker follicles) of aging wild-type mice (whole-mount view).

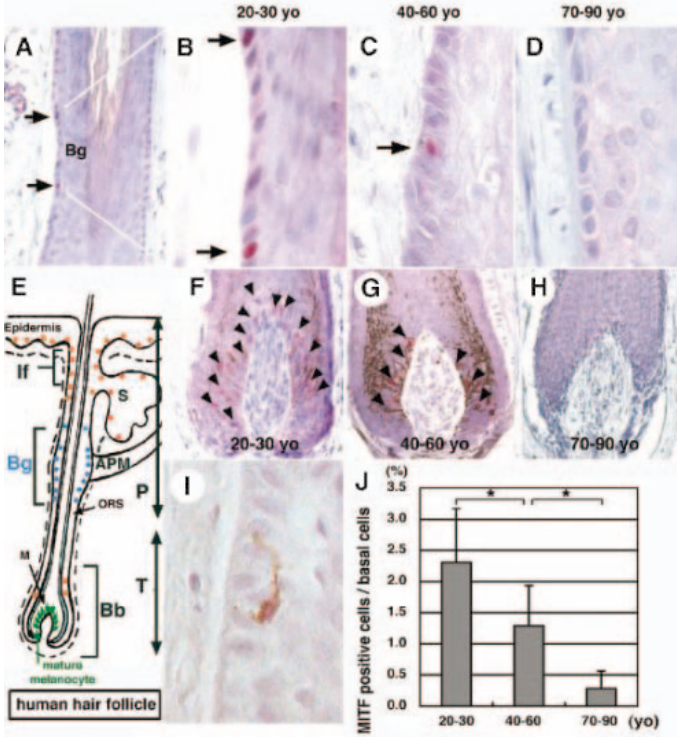

Fig. 4. Melanoblast and melanocyte distribution in human hair follicles from different age groups. Human scalp specimens were immunostained with antibodies against MITF. (**A** and **B**) MITF$^+$ cells (arrows) are distributed on the outer root sheath in the bulge of follicles from 20- to 30-year-old individuals. Magnification in (A), 200×; (B), 630×. (**C** and **D**) Representative views of the bulge from follicles of 40- to 60- and 70- to 90-year-old people, respectively (magnification, 630×). (**E**) Schematic for human hair follicle with pigmented hair. Immature MITF^{low} melanoblasts (blue) are located in the lower permanent portion (the bulge). MITF^{high} melanocytes are located in epidermis, infundibulum (If) (brown), and hair matrix (M, green). ORS, outer root sheath. See Fig. 1A for abbreviations. (**F** to **H**) The bulb region of follicles from different age groups. Mature melanocytes in the hair matrix express MITF (arrowheads). yo, years old. (**I**) An MITF$^+$ melanocyte that contains abundant melanin granules and long dendrites is detected in the bulge and subbulge of follicles specifically from middle-aged individuals. (**J**) The frequency of MITF$^+$ cells per basal keratinocytes in the bulge. Asterisks indicate statistical significance ($P < 0.01$).

Although melanin biosynthesis has been elegantly shown to be cytotoxic in the context of a certain genetic mutation (*6*), stem-cell disappearance in *Bcl2* null mice does not require melanogenesis. *Bcl2* is a transcriptional target of MITF (*30*), but *Bcl2* does not appear to fully account for melanocyte loss in the context of the weakly hypomorphic *Mitf*$^{vit/vit}$ allele.

Our data suggest a previously unknown pathophysiologic explanation for hair graying. Loss of melanocyte stem cells can be observed and temporally precedes the loss of differentiated melanocytes in the hair matrix. Thus, incomplete maintenance of melanocyte stem cells appears to cause physiologic hair graying through loss of the differentiated progeny with aging. This is associated with ectopic melanocyte pigmentation or differentiation within the niche. Possible explanations include premature differentiation or activation of a senescence program [which induces pigmentation in vitro (*31*)]. Acceleration of this process in *Mitf*^{vit} follicles implicates MITF in the self-renewal of melanocyte stem cells. The precise roles for stem-cell apoptosis versus ectopic differentiation remain to be determined but may similarly contribute to stem-cell loss in other aging organ systems.

References and Notes

1. M. A. Sussman, P. Anversa, *Annu. Rev. Physiol.* **66**, 29 (2004).
2. G. Van Zant, Y. Liang, *Exp. Hematol.* **31**, 659 (2003).
3. K. K. Wong *et al.*, *Nature* **421**, 643 (2003).
4. K. Urabe *et al.*, *Biochim. Biophys. Acta* **1221**, 272 (1994).
5. J. P. Ortonne, J. J. Nordlund, in *The Pigmentary System*, J. J. Nordlund, R. Boissy, V. J. Hearing, R. King, J. P. Ortonne, Eds. (Oxford Univ. Press, New York, 1998), pp. 489–502.
6. R. Johnson, I. J. Jackson, *Nature Genet.* **1**, 226 (1992).
7. E. K. Nishimura *et al.*, *Nature* **416**, 854 (2002).
8. F. M. Watt, B. L. M. Hogan, *Science* **287**, 1427 (2000).
9. E. Fuchs, B. J. Merrill, C. Jamora, R. DasGupta, *Dev. Cell* **1**, 13 (2001).
10. M. A. Mackenzie, S. A. Jordan, P. S. Budd, I. J. Jackson, *Dev. Biol.* **192**, 99 (1997).
11. K. R. Fitch *et al.*, *Genes Dev.* **17**, 214 (2003).
12. D. J. Veis, C. M. Sorenson, J. R. Shutter, S. J. Korsmeyer, *Cell* **75**, 229 (1993).
13. A. B. Lerner *et al.*, *J. Invest. Dermatol.* **87**, 299 (1986).
14. K. L. Rudolph *et al.*, *Cell* **96**, 701 (1999).
15. J. de Boer *et al.*, *Science* **296**, 1276 (2002); published online 11 April 2002 (10.1126/science.1070174).
16. K. Yamamura *et al.*, *Cancer Res.* **56**, 3546 (1996).
17. D. J. Tobin, R. Paus, *Exp. Gerontol.* **36**, 29 (2001).
18. E. K. Nishimura, S. R. Granter, D. E. Fisher, unpublished data.
19. R. Paus *et al.*, *J. Invest. Dermatol.* **113**, 523 (1999).
20. E. Steingrimsson, N. G. Copeland, N. A. Jenkins, *Annu. Rev. Genet.* **38**, 365 (2004).
21. E. R. Price, D. E. Fisher, *Neuron* **30**, 15 (2001).
22. W. Montagna, H. B. Chase, *Am. J. Anat.* **99**, 415 (1956).
23. R. G. Staricco, *Ann. N. Y. Acad. Sci.* **100**, 239 (1963).
24. T. Horikawa *et al.*, *J. Invest. Dermatol.* **106**, 28 (1996).
25. S. Commo, B. A. Bernard, *Pigment Cell Res.* **13**, 253 (2000).
26. J. Du *et al.*, *Am. J. Pathol.* **163**, 333 (2003).
27. S. Commo, O. Gaillard, B. A. Bernard, *Br. J. Dermatol.* **150**, 435 (2004).
28. Y. Narisawa, H. Kohda, T. Tanaka, *Acta Derm. Venereol.* **77**, 97 (1997).

29. J. Domen, S. H. Cheshier, I. L. Weissman, *J. Exp. Med.* **191**, 253 (2000).
30. G. G. McGill *et al.*, *Cell* **109**, 707 (2002).
31. E. E. Medrano *et al.*, *Mol. Biol. Cell* **5**, 497 (1994).
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Dynamic Complex Formation During the Yeast Cell Cycle

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To analyze the dynamics of protein complexes during the yeast cell cycle, we integrated data on protein interactions and gene expression. The resulting time-dependent interaction network places both periodically and constitutively expressed proteins in a temporal cell cycle context, thereby revealing previously unknown components and modules. We discovered that most complexes consist of both periodically and constitutively expressed subunits, which suggests that the former control complex activity by a mechanism of just-in-time assembly. Consistent with this, we show that additional regulation through targeted degradation and phosphorylation by Cdc28p (Cdk1) specifically affects the periodically expressed proteins.

Most research on biological networks has been focused on static topological properties (*1*), describing networks as collections of nodes and edges rather than as dynamic structural entities. Here we focus on the temporal aspects of networks, which allows us to study the dynamics of protein complex assembly during the *Saccharomyces cerevisiae* cell cycle.

Our integrative approach combines protein-protein interactions with information on the timing of the transcription of specific genes during the cell cycle, obtained from DNA microarray time series (*2*, *3*). From the latter, we derived a quality-controlled set of 600 periodically expressed genes, each assigned to the point in the cell cycle where its expression peaks (*4*). We then constructed a physical interaction network for the corresponding proteins from yeast two-hybrid screens (*5*, *6*), complex pull-downs (*7*, *8*), and curated complexes from the Munich Information Center for Protein Sequences (MIPS) database (*9*). To reduce the error rate of 30 to 50% expected in most current large-scale interaction screens (*10*, *11*), all physical interaction data were combined, a topology-based confidence score was assigned to each individual interaction [as in the STRING database (*12*)], and only high-confidence interactions were selected (*13*). These were further filtered with information on subcellular localization (*14*) to exclude interactions between proteins annotated to incompatible compartments (*13*); no curated MIPS interactions were lost because of this filtering. The topology-based scoring scheme, filtering, and extraction criteria reduced the error rate for interactions by an order of magnitude to only 3 to 5% (*13*).

In the extracted network (Fig. 1), we included, in addition to the periodically expressed ("dynamic") proteins, constitutively expressed ("static") proteins that preferentially interact with dynamic ones (*13*). The resulting network consists of 300 proteins (Fig. 1, inside circle), including 184 dynamic proteins (colored according to their time of peak expression) and 116 static proteins (depicted in white). For 412 of the 600 dynamic proteins identified in the microarray analysis, no physical interactions of sufficient reliability could be found (Fig. 1, outside circle). Some may be missed subunits of stable complexes already in the network; the majority, however, probably participate in transient interactions, which are often not detected by current interaction assays (*15*).

Although our procedure for extracting interactions might miss some cellular processes that are dominated by transient interactions, most of the stable complexes should have been captured at least partially. Tandem affinity purifications alone should identify at least half of the subunits for 87% of the known yeast complexes (*7*). Compared with the known cell cycle complexes and functional modules (*9*), we found that all but two of them were identified by our approach (better than random at $P < 10^{-30}$). The only exceptions were the anaphase-promoting complex (APC), which can only be detected with a less stringent interaction cutoff, and the Skp1p/Cullin/F-box protein complex (SCF), which appears to be the only cell cycle–related protein complex without a periodically expressed subunit. For completeness, these two complexes were added to the network. Our extraction procedure produces comparable results even if the curated MIPS complexes are excluded entirely from the analysis or if the specific extraction criteria are changed, showing that the method is robust and has much higher coverage than methods of comparable accuracy (*13*).

The derived cell cycle network (Fig. 1, inside circle) contains 29 heavily intraconnected modules; that is, complexes or groups of complex variants that exist at different time points during the yeast cell cycle. In addition to rediscovering many known cell cycle modules, our approach enables us to place more than 30 poorly characterized proteins in the cell cycle network and to predict new unexpected cell cycle contexts for other proteins (*13*). The network contains 31 isolated binary complexes, many of which involve proteins of unknown function, such as Yml119p and Yll032p, which interact and are both putative Cdc28p substrates (*16*) expressed close in time in G_2 phase (*13*).

As an example of the value of combining temporal data with protein-protein interactions, the network reveals a binary complex consisting of the uncharacterized proteins Ymr295p and Ydr348p. Because only Ydr295p is dynamic, the static protein Ydr348p can only be identified as a cell cycle–relevant protein and placed temporally through the integration of the two complementary data types. Indeed, Ydr348p is a putative Cdc28p target (*16*), and the interaction is further supported by the observation that both proteins localize to the bud neck (*14*). Virtually all complexes contain both dynamic and static subunits (Fig. 1), the latter accounting for about half of the direct interaction partners of periodically regulated proteins through all phases of the cell cycle (Fig. 2). Transcriptional regulation thus influences almost all cell cycle complexes and thereby, indirectly, their static subunits. This implies that many cell cycle proteins cannot be identified through the analysis of any sin-

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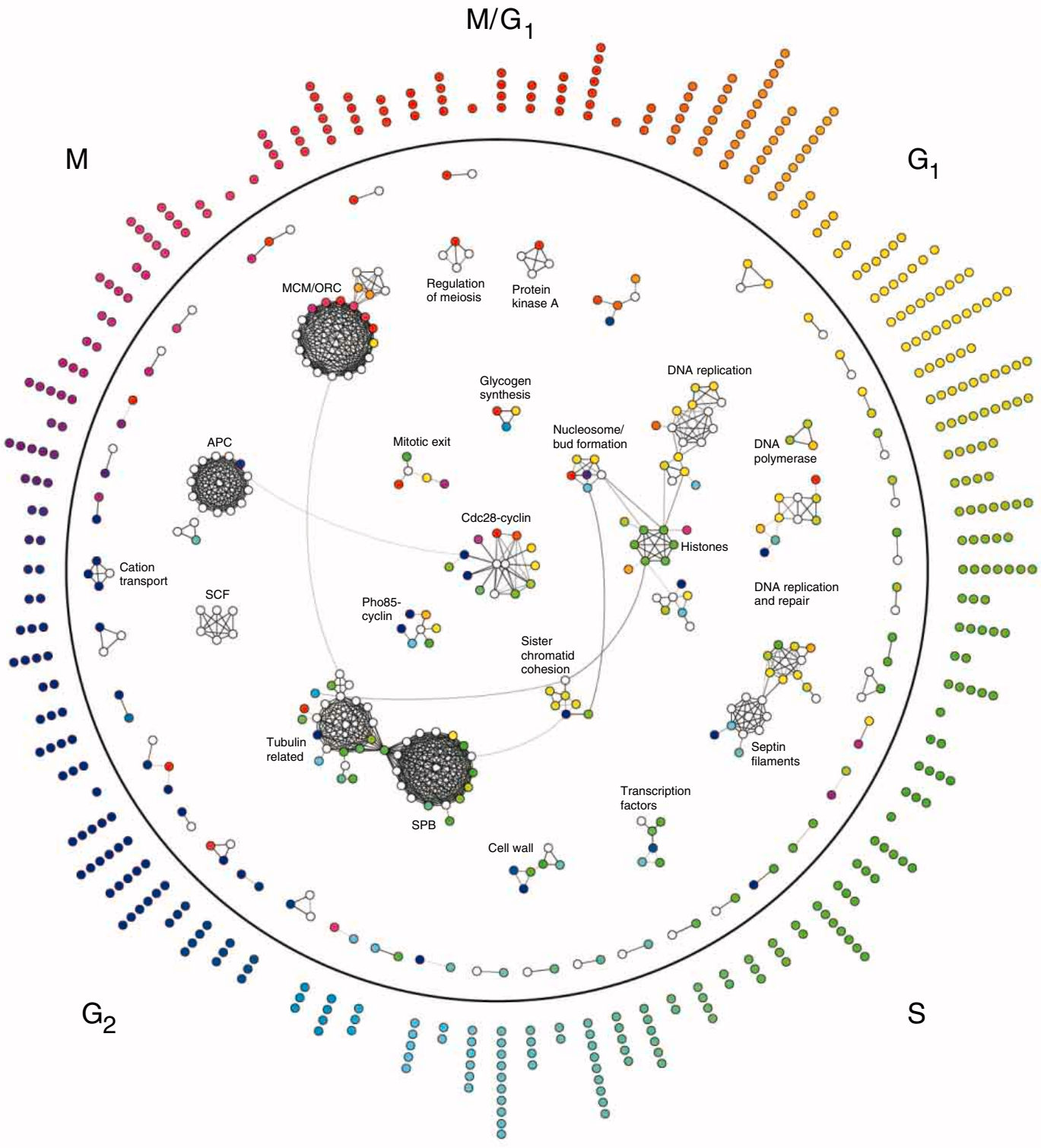

Fig. 1. Temporal protein interaction network of the yeast mitotic cell cycle. Cell cycle proteins that are part of complexes or other physical interactions are shown within the circle. For the dynamic proteins, the time of peak expression is shown by the node color; static proteins are represented by white nodes. Outside the circle, the dynamic proteins without interactions are both positioned and colored according to their peak time and thus also serve as a legend for the color scheme in the network. More detailed versions of this figure (including all protein names) and the underlying data are available online at www.cbs.dtu.dk/cellcycle.

gle type of experimental data but only through integrative analysis of several data types.

In addition to suggesting functions for individual proteins, the network (Fig. 1) indicates the existence of entire previously unknown modules. Most notably, the network reveals a module that includes two poorly characterized proteins (Nis1p and Yol070p) and links processes related to the nucleosomes with mitotic events in the bud (Fig. 3A) (*13*).

Transcription of cell cycle–regulated genes is generally thought to be turned on when or just before their protein products are needed: often referred to as just-in-time synthesis. Contrary to the cell cycle in bacteria (*17*), how-

ever, just-in-time synthesis of entire complexes is rarely observed in the network. The only large complex to be synthesized in its entirety just in time is the nucleosome, all subunits of which are expressed in S phase to produce nucleosomes during DNA replication.

Instead, the general design principle appears to be that only some subunits of each complex are transcriptionally regulated in order to control the timing of final assembly. Several examples of this just-in-time assembly (rather than just-in-time synthesis) are suggested by the network, including the prereplication complex (Fig. 3B), complexes involved in DNA replication and repair, the spindle pole body, proteins related to the cytoskeleton, and numerous smaller complexes or modules (*13*). We find that the transcriptome time mappings visualized in Fig. 1 are in close agreement with previous studies on the dynamic formation of individual protein complexes, suggesting that the timing of transcription of dynamic proteins is indicative of the timing of assembly and action of the complexes and modules (Fig. 3) (*13*). Just-in-time assembly would have an advantage over just-in-time synthesis of entire complexes in that only a few components need to be tightly regulated in order to control the timing of final complex assembly. This would explain the recent observation that the periodic transcription of specific cell cycle genes is poorly conserved through evolution (*18*). For the prereplication complex, exactly this variation between organisms has been shown, although the subunits and the order in which they assemble are conserved (*19*).

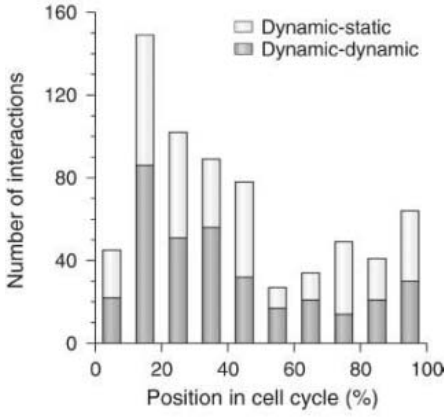

Fig. 2. Interactions during the cell cycle. The number of interactions of dynamic proteins with other dynamic proteins (dynamic-dynamic) and with static proteins (dynamic-static) is shown as a function of cell cycle progression. Zero time corresponds to the time of cell division. The large number of interactions during G_1 phase reflects a general overrepresentation of genes expressed in this part of the cell cycle (Fig. 1).

Dynamic complex assembly also functions as a mechanism for temporal regulation of substrate specificity, best exemplified by the association of the cyclin-dependent kinase Cdc28p with its various transcriptionally regulated cyclins and inhibitors (Fig. 3C). Our approach accurately reproduces this key regulatory system and its temporal dynamics, correctly placing each of the Cdc28p interactions' partners at their time of function and capturing even very transient interactions such as phosphorylation and ubiquitination (Fig. 3C). Transient interactions are also captured between cyclins of the Pho85p system and two M-phase–specific proteins, Swi5p and Mmr1p. Swi5p has already been identified as a Pho85p-Pcl2p substrate (*20*); from the network context, we propose that the uncharacterized phosphoprotein Mmr1p is a target of the Pho85p-Pcl7p kinase (*13*). The cell cycle role of Mmr1p is supported by binding of the transcription factors Fkh2p, Mcm1, and Ndd1p, which cooperatively activate transcription at the G_2/M transition, exactly when the expression of Mmr1p peaks (*21*, *22*).

Furthermore, we used the network model to determine whether the transcriptionally regulated subunits are also regulated by phosphorylation. The 332 putative Cdc28p targets identified in a recent screen (*16*) are indeed much more frequent among the dynamic than among the static proteins in the network [significant at $P < 10^{-4}$ (*13*)]. In the cell cycle network (Fig. 1), 27% of the periodically transcribed proteins are putative Cdc28p targets, in comparison to only 8% of the static proteins and 6% of all yeast proteins. This link between transcriptional and posttranslational control probably reflects multiple roles: It allows the control of complex assembly by dynamic proteins to be fine-tuned, provides new nonmodified Cdc28p targets in each cell cycle, and regulates the degradation of the dynamic proteins through phosphorylation. The latter is supported by an overrepresentation of predicted PEST (regions rich in Pro, Glu, Ser, and Thr) degradation signals among both the dynamic proteins and the putative Cdc28p targets in the network [significant at $P < 10^{-2}$ and $P < 10^{-3}$, respectively (*13*)]. Cell cycle–regulated transcription thus supplies the cell with nonmodified complex components at the time of complex assembly, many of which are later phosphorylated and specifically targeted for degradation.

With the emergence of new large-scale data sets, including assays focused on protein-DNA interactions and transient protein-protein interactions, we expect more of the dynamic proteins to be included in the network. Also, we currently lack information about the life-

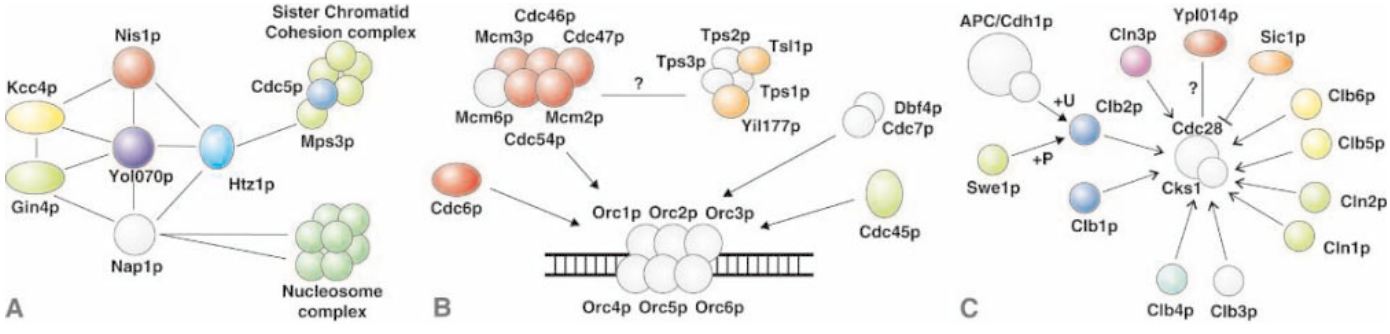

Fig. 3. Dynamic modules. Each panel shows a specific module from the network in Fig. 1, using the corresponding colors of individual proteins. (**A**) Previously unknown module connecting processes related to chromosome structure with mitotic events in the bud. The nucleosome assembly protein Nap1p is known to shuttle between the nucleus and cytosol and regulates the activity of Gin4p, one of two G_1-expressed budding-related kinases in the module. Nap1p and the histone variant Htz1p connect the module to the nucleosome and sister chromatid modules, respectively. Two poorly characterized proteins, Nis1p and the putative Cdc28p substrate Yol070p (*16*), are both expressed in mitosis and localize to the bud neck (*13*). (**B**) Schematic representation of the dynamic assembly of the prereplication complex. It contains six static proteins (Orc1p to Orc6p) that are bound to origins of replication throughout the entire division cycle. A subcomplex of six Mcm proteins is recruited to the ORC complex in G_1 phase by a Cdc6p-dependent mechanism; we see the corresponding genes (except *MCM6*) transcribed just before that. Final recruitment of the replication machinery is dependent on Cdc45p, which we find expressed in early S phase. More details and case stories are available (*13*). (**C**) Cdc28p module, with the different cyclins and interactors placed at their time of synthesis. At the end of mitosis, the cyclins are ubiquitinated and targeted for destruction by APC and SCF, reflected in the network by the interaction between Cdh1p and Clb2p. The latter also interacts with Swe1p, which inhibits entry into mitosis by phosphorylating Cdc28p in complex with Clb-type cyclins. We also discovered an uncharacterized protein, Ypl014p, in this well-studied module (*13*).

time of the observed complexes and modules. With reliable time series of protein abundances, preferably in individual compartments, the resolution of this temporal network can be increased considerably, because even individual interactions over time could then be monitored. Moreover, the integrative approach presented here should be applicable to any biological system for which both interaction data and time series are available.

References and Notes

1. A. L. Barabasi, Z. N. Oltvai, *Nature Rev. Genet.* **5**, 101 (2004).
2. R. J. Cho *et al.*, *Mol. Cell* **2**, 65 (1998).
3. P. T. Spellman *et al.*, *Mol. Biol. Cell* **9**, 3273 (1998).
4. U. de Lichtenberg *et al.*, *Bioinformatics* doi:10.1093/bioinformatics/bti093 (2004).
5. T. Ito *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 1143 (2000).
6. P. Uetz *et al.*, *Nature* **403**, 623 (2000).
7. A.-C. Gavin *et al.*, *Nature* **415**, 141 (2002).
8. Y. Ho *et al.*, *Nature* **415**, 180 (2002).
9. H. W. Mewes *et al.*, *Nucleic Acids Res.* **32**, 5539 (2004).
10. A. M. Edwards *et al.*, *Trends Genet.* **18**, 529 (2002).
11. C. von Mering *et al.*, *Nature* **417**, 399 (2002).
12. C. von Mering *et al.*, *Nucleic Acids Res.* **31**, 258 (2003).
13. See supporting information on *Science* Online for details.
14. K. R. Christie *et al.*, *Nucleic Acids Res.* **32**, D311 (2004).
15. L. J. Jensen, P. Bork, *Drug Discovery Today: TARGETS* **3**, 51 (2004).
16. J. A. Ubersax *et al.*, *Nature* **425**, 859 (2003).
17. H. H. McAdams, L. Shapiro, *Science* **301**, 1874 (2003).
18. G. Rustici *et al.*, *Nature Genet.* **36**, 809 (2004).
19. M. L. DePamphilis, *Gene* **310**, 1 (2003).
20. V. Measday, H. McBride, J. Moffat, D. Stillman, B. Andrews, *Mol. Microbiol.* **35**, 825 (2000).
21. I. Simon *et al.*, *Cell* **106**, 697 (2001).
22. T. I. Lee *et al.*, *Science* **298**, 799 (2002).

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Escape of Intracellular *Shigella* from Autophagy

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The degradation of undesirable cellular components or organelles, including invading microbes, by autophagy is crucial for cell survival. Here, *Shigella*, an invasive bacteria, was found to be able to escape autophagy by secreting IcsB by means of the type III secretion system. Mutant bacteria lacking IcsB were trapped by autophagy during multiplication within the host cells. IcsB did not directly inhibit autophagy. Rather, *Shigella* VirG, a protein required for intracellular actin-based motility, induced autophagy by binding to the autophagy protein, Atg5. In nonmutant *Shigella*, this binding is competitively inhibited by IcsB binding to VirG.

During the multiplication of microbes within host cells, bacteria become sequestered in membrane-bound organelles such as phagosomes (*1–3*). This event is a key component of host defense against invading microbes. Nevertheless, some invasive bacteria such as *Legionella*, *Salmonella*, *Mycobacteria*, and *Brucella* can block or alter the maturation of the phagosome and can reside in vacuoles (*2–7*). Some others such as *Shigella* (*8*, *9*), *Listeria monocytogenes* (*10*), and *Rickettsia conorii* (*11*) can escape from phagosomes into the cytoplasm, multiply, and disseminate into neighboring cells by eliciting actin polymerization. Cytoplasmic pathogens may thus circumvent autophagic events.

IcsB, one of the *Shigella flexneri* effectors, is secreted by means of the type III secretion system (TTSS) of cytoplasmic bacteria and located on the bacterial surface (*12*). The *icsB* mutant is fully invasive and able to escape from the vacuole but is defective in spreading within host cells (*12*).

To clarify the role of IcsB in promoting infection, we investigated the intracellular behaviors of the *icsB* mutant (Δ*icsB*), YSH6000 (wild type; WT), and Δ*icsB*/pIcsB (the *icsB* complement strain). In baby hamster kidney (BHK) cells, although mutants lacking IcsB multiplied as normal for about 3 hours, their growth plateaued 4 hours after invasion (fig. S1A). To characterize intracellular bacteria, we introduced green fluorescent protein plasmid (pGFP) into Δ*icsB* and WT then investigated BHK cells infected with bacteria 4 hours after infection. Δ*icsB*/pGFP colocalized with markers for acidic lysosomes (Lysotracker) or autophagosomes [monodancyl-cadaverin (MDC)], where the bacterial morphology was indistinct. (fig. S1, B and C). WT cells, on the whole, did not colocalize with the same markers: 37.2% of Δ*icsB* bacteria colocalized with lysosomes compared with only 10.2% of WT. Furthermore, when BHK cells expressing GFP-LC3, an autophagosome-specific marker (*13*, *14*), were infected with Δ*icsB* or WT, ~40% of Δ*icsB* was associated with LC3 signal; bacterial shape was also indistinct compared with WT (fig. S1D). To further characterize the Δ*icsB* defect, we exploited MDCK cells (epithelial cells from dog kidney) expressing GFP-LC3 (MDCK/pGFP-LC3 cells), which made it feasible to visualize cytoplasmic organelles and bacteria (Fig. 1A). The number of LC3-positive Δ*icsB* was greater than that of WT throughout the 1 to 6 hours after infection. The LC3-positive population of Δ*icsB* had increased 50% by 6 hours, whereas that of WT remained at 10 to 15% (Fig. 1B). Two hours after infection, WT and Δ*icsB* had similar numbers of actin tails. After 4 hours, however, the population was decreased in Δ*icsB* (fig. S2), presumably because Δ*icsB* was within autophagosomes. The LC3-positive population of the Δ*icsB*/pIcsB was decreased: it fell to a level as low as that of WT (Fig. 1B). Autophagic events can be triggered by amino acid starvation (*13*). MDCK/pGFP-LC3 cells were infected with Δ*icsB* or WT, under amino acid–starved conditions. LC3-positive bacteria in MDCK cells were significantly increased from 10 to 16% (WT) and from 23 to 36% (Δ*icsB*) in response to amino acid deprivation (fig. S3). Conversely, when MDCK cells were treated with known inhibitors of autophagy or of lysosomes, such as Wortmannin, 3-methyladenine (3-MA) or bafilomycin-A1 (Baf-A1), the LC3-positive Δ*icsB* population was markedly decreased (Fig. 1, C and D). In the presence of Baf-A1, fusion of lysosomes with autophagosomes containing Δ*icsB* was blocked, which would have allowed the bacteria to escape into the cytosol. Consistently, despite the smaller diameter (<0.15 mm) of plaques formed by Δ*icsB* 2 days after infection than that of plaques formed by WT (~0.5 mm), the plaque-forming capacity of Δ*icsB* was restored by treatment with Baf-A1 (fig. S4). Another investigation was made in *atg5*-knockout mouse embryonic fibroblasts

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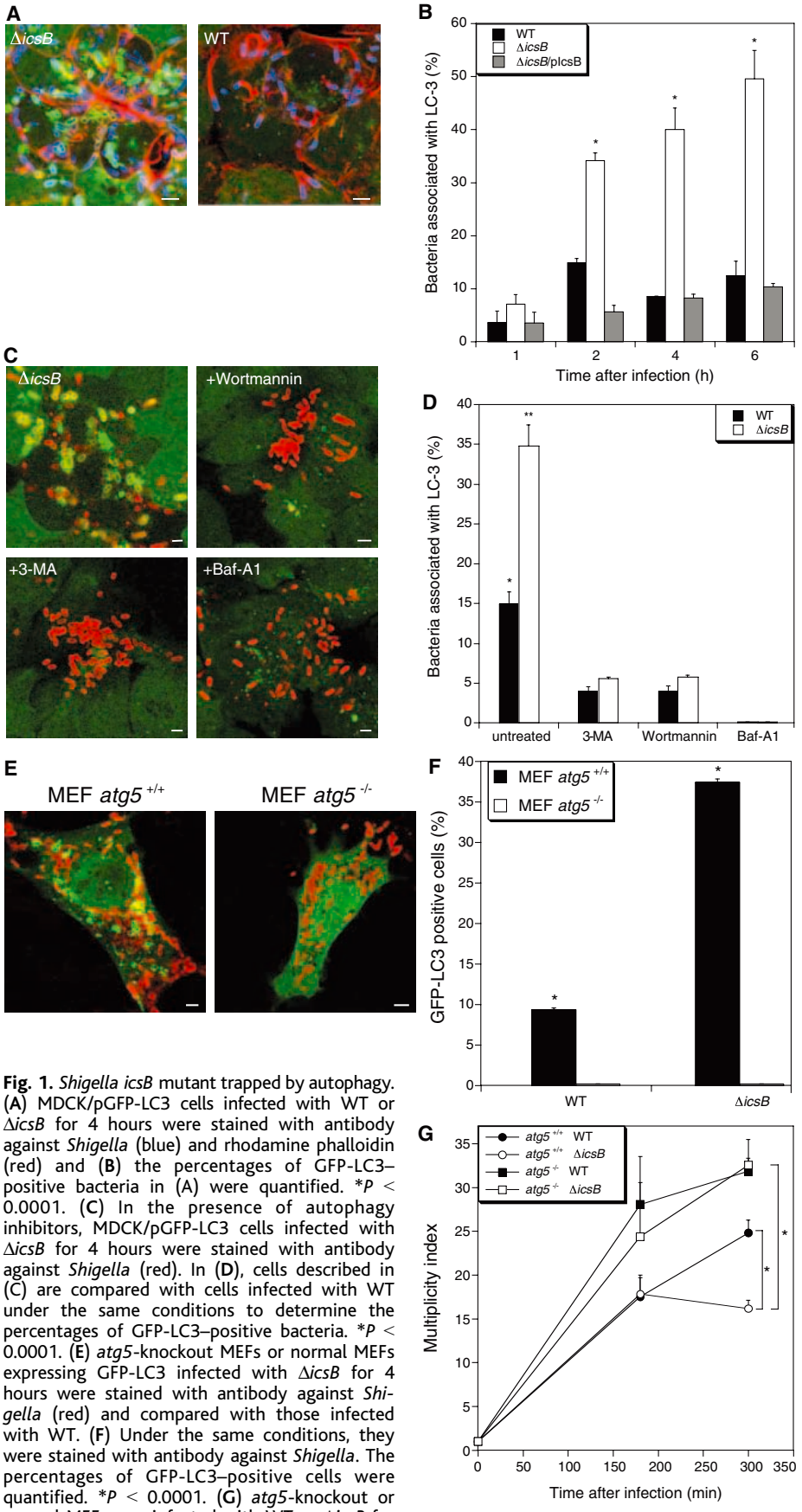

Fig. 1. *Shigella icsB* mutant trapped by autophagy. (**A**) MDCK/pGFP-LC3 cells infected with WT or Δ*icsB* for 4 hours were stained with antibody against *Shigella* (blue) and rhodamine phalloidin (red) and (**B**) the percentages of GFP-LC3–positive bacteria in (A) were quantified. **P* < 0.0001. (**C**) In the presence of autophagy inhibitors, MDCK/pGFP-LC3 cells infected with Δ*icsB* for 4 hours were stained with antibody against *Shigella* (red). In (**D**), cells described in (C) are compared with cells infected with WT under the same conditions to determine the percentages of GFP-LC3–positive bacteria. **P* < 0.0001. (**E**) *atg5*-knockout MEFs or normal MEFs expressing GFP-LC3 infected with Δ*icsB* for 4 hours were stained with antibody against *Shigella* (red) and compared with those infected with WT. (**F**) Under the same conditions, they were stained with antibody against *Shigella*. The percentages of GFP-LC3–positive cells were quantified. **P* < 0.0001. (**G**) *atg5*-knockout or normal MEF were infected with WT or Δ*icsB* for indicated periods. Intracellular multiplication of bacteria is determined. **P* < 0.0001. Scale bars (A), (B), and (E), 2 μm.

(*atg5*$^{-/-}$ MEFs), which are defective in autophagy. When *atg5*$^{-/-}$ MEFs or normal MEF cells (*atg5*$^{+/+}$ MEFs) expressing GFP-LC3 were infected with Δ*icsB*, even though LC3-positive Δ*icsB* is detectable in normal MEFs, signals were barely detected in the *atg5*-knockout MEFs (Fig. 1, E and F). Consistently, intracellular growth of Δ*icsB* was recovered to the level of WT in *atg5*$^{-/-}$ MEF cells (Fig. 1G), which provided further evidence that the defective intracellular phenotype of Δ*icsB* was associated with autophagy.

MDCK cells infected with Δ*icsB* or WT for 4 hours were examined by thin-section electron microscopy (EM). Δ*icsB* was frequently enclosed by lamellar membranous structures, in striking contrast to the phagocytic membrane surrounding an invading bacterium (Fig. 2A). Occasionally, some bacteria enclosed by lamellar membranes were also surrounded by structures like onion skin (Fig. 2A, arrows). Six hours after invasion, some bacteria enclosed by an onion skin–like structure had become indistinct (fig. S5A). At the same stage of infection, WT bacteria generally lacked the lamellar membranes. Instead, most had long actin tails, as seen in motile bacteria (fig. S5B). To confirm that the lamellar membranes surrounding Δ*icsB* were autophagic membranes, MDCK/pGFP-LC3 cells infected with Δ*icsB* 4 hours after infection were analyzed by immunogold EM with antibody against GFP. The lamellar membrane around Δ*icsB* in MDCK/pGFP-LC3 cells was specifically labeled with immunogold (Fig. 2B, arrows). Onion skin–like membranous structures associated with the bacterium were also highly labeled with immunogold (Fig. 2B, arrowheads). Thus intracellular *Shigella* lacking IcsB readily succumbs to autophagy within epithelial cells.

To explore the role of IcsB, we assessed whether IcsB is linked to autophagic proteins. We constructed COS-7 transfectants expressing GFP-Atg5, GFP-Beclin (Atg6), GFP-LC3 or Myc in complex with the vacuolar protein-sorting protein Vps34, that is Myc-Vps34 (type III phosphatidylinositol 3-kinase), and each cell lysate was placed in a pull-down assay with glutathione *S*-transferase (GST) in complex with IcsB. However, under these experimental conditions, none was precipitated by GST-IcsB. Intriguingly, we noted that the LC3 and Atg5 signals in the vicinity of the bacterial body of Δ*icsB* tended to be distributed asymmetrically, with signals occasionally accumulating at one pole of the bacterium. Furthermore, lamellar membranes associated with Δ*icsB* in EM were also frequently seen in the area at one end of the bacterial body, which reminded us of the asymmetric distribution of VirG (IcsA) on *Shigella*

required for actin-based intracellular motility (*15*). We tested whether some autophagic component(s) might directly associate with VirG and so possibly trigger the development of autophagy around the bacterium. Cell lysates prepared from COS-7 cells expressing GFP-Atg5, GFP-Beclin, GFP-LC3, or Myc-Vps34 were pulled down with GST-VirGα1 (the surface-exposed VirG portion) (*16*), and the bound proteins were analyzed by immunoblotting. Note that only Atg5 was reproducibly precipitated with GST-VirGα1 (fig. S6A). Furthermore, the binding capacity was also confirmed by performing immunoprecipitation experiments in 293T cells expressing Atg5-Myc and GFP-VirGα1 (fig. S6B), which raised the possibility that Atg5 might have some affinity for VirG. To pursue this, BHK cells expressing GFP-Atg5 (BHK/pGFP-Atg5) were infected with Δ*icsB* for 4 hours and investigated for association of the bacterium with GFP-Atg5. The GFP-Atg5 signals were occasionally confined to one pole of the bacterium. It is noteworthy that when BHK/pGFP-Atg5 cells were infected with *virG* mutant (Δ*virG*), the association of the Atg5 signal was barely detectable, but the signal was restored after infection with Δ*virG*/pVirG (Fig. 3A). The Apg5 signal occasionally associated with VirG at one pole of the bacterium (Fig. 3B).

To verify the concept, MDCK/pGFP-LC3 cells infected with WT, Δ*icsB*, Δ*virG*, Δ*virG*/pVirG, or Δ*virG*/Δ*icsB* (double mutant) were also examined for association of the LC3 signal with the bacteria. LC3-positive Δ*virG* accounted for only 1.5%, whereas LC3-positive Δ*icsB* made up 37% (Fig. 3C). Although LC3-positive Δ*virG*/pVirG was increased to 23%, exceeding that from WT (15%), LC3-positive Δ*virG*/Δ*icsB* was still less than 2% (Fig. 3C). Consistently, similar results were obtained from the experiment using normal MEFs expressing GFP-LC3 (fig. S7, A and B). Then, we investigated the multiplicity of WT, Δ*icsB*, Δ*virG*, and Δ*virG*/Δ*icsB* within BHK cells. Δ*virG* grew slightly better than WT within cells and found that the deficiency of intracellular growth of Δ*icsB* was recovered in Δ*virG*/Δ*icsB*, which suggests that VirG is critical for *Shigella* to trigger autophagy (fig. S8). Atg5 may play a role as the "seed" of isolation membranes in the autophagic process (*17*). VirG present on *Shigella* could thus be the target for autophagy, in which IcsB would apparently act as the anti-autophagocytic agents. To assess this possibility, immobilized GST-VirGα1 with or without IcsB was incubated with a lysate of 293T cells expressing Atg5-Myc, and the bound proteins were examined by immunoblotting. IcsB was examined in a pull-down assay with GST-VirGα1 in the presence of Atg5; Atg5-Myc was precipitated in a pull-down assay only when IcsB was absent (Fig. 3D). The extent of His-Atg5 binding to GST-VirGα1 1 decreased as we raised the amount of purified IcsB added, which suggests that Atg5 binding to VirG was inhibited by IcsB in a dose-dependent manner (Fig. 3E). To define the VirG region involved in binding, various truncated VirGα1 versions (VirGα1 through to VirGα8) (*16*) were tested for the ability to bind to either His-Atg5 or IcsB by using the GST-VirG pull down assay. Both His-Atg5 and IcsB were pulled down by the same series of VirG truncations, which suggests that they share the amino acid residues 320 through 433 of VirG, which are involved in binding (Fig. 3F). Indeed, in MDCK/pGFP-LC3 cells infected with Δ*virG*/pD10-VirG2 (pVirG lacking VirG internal amino acid residues 319 to 507) but not Δ*virG*/pVirG (full-length VirG), the GFP-LC3 signals were barely visible around bacteria (Fig. 3G). Thus the interaction of IcsB with VirG by means of the putative VirG targeting sequence for Atg5 might be a mechanism for eluding the development of autophagy in the vicinity of the bacterial surface in mammalian cells.

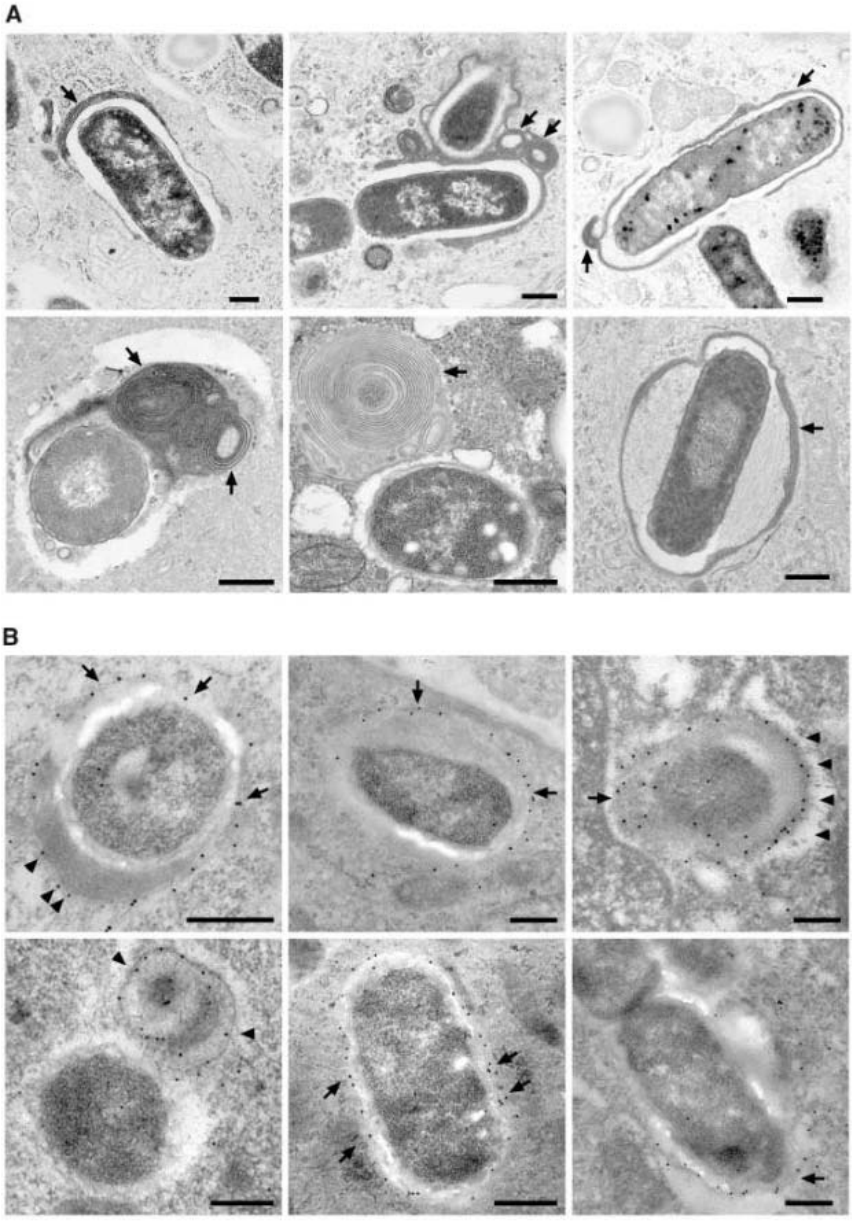

Fig. 2. Multilamellar structures enclosing Δ*icsB* in MDCK cells. (**A**) MDCK cells infected with Δ*icsB* were observed by EM 4 hours later. Multilamellar structures surround the bacterium (arrows). (**B**) GFP-LC3 (arrows) is localized in the multilamellar structures (arrowheads) surrounding the bacterium, under immunogold EM. Scale bars, 0.5 μm.

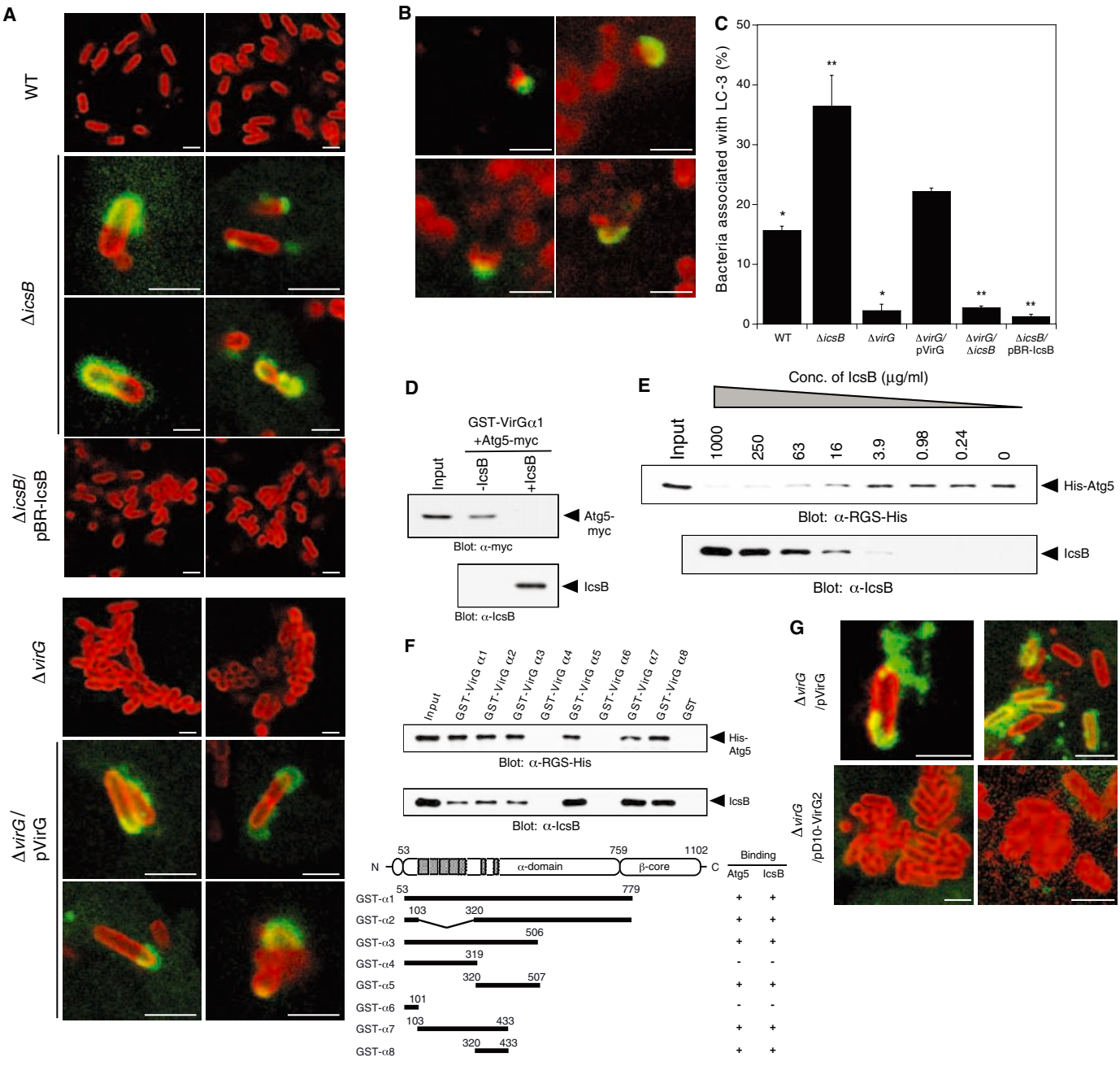

Fig. 3. VirG affinity for Apg5 is inhibited by IcsB. **(A)** BHK/pGFP-Atg5 cells infected with indicated *Shigella* strains for 4 hours were stained with antibody against *Shigella* (red). **(B)** BHK/pGFP-Atg5 cells infected with Δ*virG*/pVirG for 4 hours were stained with antibody against VirG (red). **(C)** MDCK/pGFP-LC3 cells infected with indicated *Shigella* strains for 4 hours were stained with antibody against *Shigella*. The percentages of GFP-LC3–positive bacteria were quantified. $*P < 0.0001$. **(D)** Analysis of IcsB inhibition for Atg5 binding to GST-VirGα1 with the use of cleared lysates of 293T cells expressing Atg5-Myc. **(E)** Dose-response analysis of IcsB inhibition for Atg5 binding to GST-VirGα1. **(F)** Determination of VirG domain involved in binding with Atg5 and IcsB (top) and schematic representation of the virGα1 derivatives (bottom). **(G)** MDCK/pGFP-LC3 cells infected with Δ*virG*/pVirG or Δ*virG*/pD10-VirG2 for 4 hours were stained with antibody against *Shigella* (red). Scale bars (A), (B), and (G), 2 μm.

To demonstrate whether VirG itself was a target for autophagy, BHK cells expressing Myc-LC3 (BHK/pMyc-LC3) were infected with an *E. coli* K-12 strain expressing VirG plus GFP (*E. coli*/pSU-GFP/pUC-VirG) or GFP (*E. coli*/pSU-GFP) together with Δ*virG*/Δ*icsB* (invasion-positive but autophagy-negative *S. flexneri* used as the carrier, which allowed *E. coli* to move into the host cytoplasm). The association with LC3 was investigated. In cells infected with *E. coli*/pSU-GFP/pUC-VirG but not *E. coli*/pSU-GFP, bacterial signals were localized with Myc-LC3 signals, and the VirG-associated signals merged with the GFP-LC3 signals (Fig. 4). It is thus highly likely that VirG presented on the *Shigella* surface in the host cytoplasm triggers autophagy.

Shigella VirG can thus be targeted for autophagy perhaps through its affinity for Atg5, and IcsB can interfere with this autophagic process. The role of IcsB in bacterial infection differs from other known TTSS-secreted bacterial effectors, in that its role is to camouflage its own bacterial target molecule (VirG) from the autophagic host defense system.

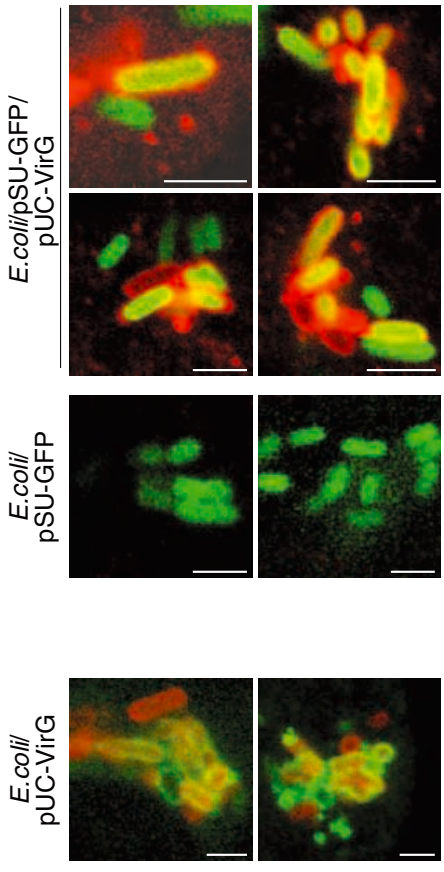

Fig. 4. VirG triggers autophagy. BHK/pMyc-LC3 coinfected with *E. coli*/pSU-GFP/pUC-VirG and Δ*virG*/Δ*icsB* at a ratio of 10:1 for 2 hours were stained with antibody against Myc (red) (**top**). As a control, *E. coli*/pSU-GFP was used. (**Bottom**) BHK/pGFP-LC3 cells were coinfected with *E. coli*/pUC-VirG and Δ*virG*/Δ*icsB* as described above, and stained with antibody against VirG (red) (bottom). Scale bar, 2 μm. Note that as *E. coli*/pUC-VirG expressed a high level of surface VirG, the VirG signal was visible over the entire bacterial surface.

References and Notes

1. K. A. Rich, C. Burkett, P. Webster, *Cell. Microbiol.* **5**, 455 (2003).
2. B. R. Dorn, W. A. Dunn Jr., A. Progulske-Fox, *Cell. Microbiol.* **4**, 1 (2002).
3. S. Méresse *et al.*, *Nature Cell Biol.* **1**, E183 (1999).
4. L. D. Hernandez, M. Pypaert, R. A. Flavell, J. E. Gálan, *J. Cell Biol.* **163**, 1123 (2003).
5. K. Kirkegaard, M. P. Taylor, W. T. Jackson, *Nature Rev. Microbiol.* **2**, 301 (2004).
6. G. Mariño, C. López-Otín, *Cell. Mol. Life Sci.* **61**, 1439 (2004).
7. A. M. Cuervo, *Trends Cell Biol.* **14**, 70 (2004).
8. M. L. Bernardini *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3867 (1989).
9. P. Cossart, P. J. Sansonetti, *Science* **304**, 242 (2004).
10. L. G. Tilney, D. A. Portnoy, *J. Cell Biol.* **109**, 1597 (1989).
11. E. Gouin *et al.*, *Nature* **427**, 457 (2004).
12. M. Ogawa *et al.*, *Mol. Microbiol.* **48**, 913 (2003).
13. Y. Kabeya *et al.*, *EMBO J.* **19**, 5720 (2000).
14. T. Yoshimori, *Biochem. Biophys. Res. Commun.* **313**, 453 (2004).
15. J. R. Robbins *et al.*, *Mol. Microbiol.* **41**, 861 (2001).
16. T. Suzuki, S. Saga, C. Sasakawa, *J. Biol. Chem.* **271**, 21878 (1996).
17. N. Mizushima *et al.*, *J. Cell Biol.* **152**, 657 (2001).

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Materials and Methods
Figs. S1 to S8
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Nod2-Dependent Regulation of Innate and Adaptive Immunity in the Intestinal Tract

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The gene encoding the Nod2 protein is frequently mutated in Crohn's disease (CD) patients, although the physiological function of Nod2 in the intestine remains elusive. Here we show that protective immunity mediated by Nod2 recognition of bacterial muramyl dipeptide is abolished in Nod2-deficient mice. These animals are susceptible to bacterial infection via the oral route but not through intravenous or peritoneal delivery. Nod2 is required for the expression of a subgroup of intestinal anti-microbial peptides, known as cryptdins. The Nod2 protein is thus a critical regulator of bacterial immunity within the intestine, providing a possible mechanism for Nod2 mutations in CD.

Homozygous mutations in Nod2, a member of the nucleotide-binding oligomerization domain–leucine-rich repeat (NOD-LRR) family of proteins, are highly correlated with the incidence of Crohn's disease (CD), which suggests that Nod2 plays an important role in intestinal immunity (*1*, *2*). These CD-associated genetic variants are deficient in their ability to sense muramyl dipeptide (MDP), a conserved structure in bacterial peptidoglycan that is normally recognized by Nod2 (*3*–*5*). Consistent with these functional studies, homozygosity and compound heterozygosity increase the relative risk of developing CD by as much as 40-fold as compared to simple heterozygosity (~2- to 4-fold) (*1*, *2*, *6*). Nevertheless, the physiological role of Nod2 in intestinal immunity remains unclear.

To assess the function of Nod2, we generated Nod2$^{-/-}$ mice using a targeting construct to replace the NOD, which is essential for activation of the protein (fig. S1, A and B) (*7*). Nod2$^{-/-}$ mice were outwardly healthy and displayed normal lymphoid and myeloid cellular composition in the thymus and spleen (fig. S2). The animals also displayed no overt symptoms of intestinal inflammation when observed for up to 6 months, which was confirmed by histological examination (fig. S3), and there was no significantly enhanced susceptibility to colitis in the dextran sulfate–induced model (fig. S4).

Detection of MDP by Nod2 was confirmed by stimulation of wild-type and Nod2$^{-/-}$ bone marrow–derived macrophages (BMDMs) with the following Toll-like receptor (TLR) agonists: lipopolysaccharide (LPS, TLR4 ligand); Pam3CS(K)$_4$ (synthetic lipopeptide, TLR2 ligand); and double-stranded RNA [polyinosinic:polycytidylic acid (poly I:C), TLR3 ligand] in the presence or absence of MDP (*8*–*12*). The synergistic effect of MDP and TLR ligands for the production of interleuken-6 (IL-6) and IL-12 p40 in wild-type macrophages was dependent on the dose of MDP added (Fig. 1, A and B, and fig. S5F) and was absent in Nod2$^{-/-}$ macrophages, revealing that Nod2 is required for the detection of MDP in macrophages. The stimulation of wild-type macrophages with MDP, which results in the activation of nuclear factor κB (NF-κB), p38, and extracellular signal–regulated kinase (ERK), was strongly reduced in Nod2$^{-/-}$ macrophages, again indicating that Nod2 is required for MDP signaling (Fig. 1C). In light of the expression of Nod2 in CD40^{+}/CD86^{+} dendritic cells (DCs) (*13*), we tested the potential involvement of the protein in the detection of MDP by DCs. Although immature bone marrow–derived dendritic cells (BMDCs) from wild-type mice were capable

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of secreting the proinflammatory cytokines IL-6 and tumor necrosis factor–α (TNF-α), these cytokines were not detectable from Nod2$^{-/-}$ immature BMDCs (Fig. 1D). MDP alone did not induce cytokine production from BMDMs.

Previous studies have shown that pretreatment with MDP sensitizes mice to endotoxic shock induced by LPS injection (*14*). Although pretreatment with 250 μg of MDP and subsequent highly purified LPS injection were fatal in wild-type mice by day 6, Nod2$^{-/-}$ mice were resistant to LPS challenge (Fig. 1E). LPS injection without MDP priming resulted in the similar survival of both wild-type and Nod2$^{-/-}$ animals. These indicate an essential role for Nod2 in detecting MDP in vivo.

Nod2 has recently been proposed to serve as a negative regulator of TLR2 signaling in generating the Th1 phenotype (*15*). In testing the response of Nod2$^{-/-}$ BMDMs for the production of inflammatory cytokines, including IL-6, IL-12 p40, TNF-α, and IL-1β, after stimulation of various TLR ligands, including TLR2 ligands (fig. S5, A to E), we failed to observe any significant diminution in the response of Nod2$^{-/-}$ macrophages to either LPS and TLR2 ligands (fig. S5, A to C). We also observed a synergistic effect of lipopeptide Pam3CS(K)$_4$ (TLR2 ligand) and MDP in wild-type macrophages but not in Nod2$^{-/-}$ macrophages (fig. S5F). These results suggest that any potential negative role of Nod2 in the TLR2 response is not a universal phenomenon. Determination of whether there is a contribution to TLR2 response from specific experimental details, genetic background, or cell types examined will require further study.

In light of previous studies showing the role of TLRs in the adaptive immune system (*16*), we immunized wild-type and Nod2$^{-/-}$ mice with MDP (100 μg per mouse) and with human serum albumin (HSA) (50 μg per mouse) by intraperitoneal injection. Three weeks later, we boosted with MDP (20 μg per mouse) and HSA (10 μg per mouse). Serum samples were obtained 2 weeks after the first immunization and 1 week after the boost, and antigen-specific serum immunoglobulin (Ig) was assessed by enzyme-linked immunosorbent assay (ELISA). Although HSA-specific antibodies were detected 2 weeks after immunization in wild-type mice, Nod2$^{-/-}$ animals displayed a severe deficiency in the production of antigen-specific Ig, specifically in IgG1, which is the predominant Nod2-dependent isotype (Fig. 2, A and B). The defect in antigen-specific Ig production was observed even after boosting mice with MDP and HSA. Nod2$^{-/-}$ mice failed to produce HSA-specific Ig and IgG1 (Fig. 2, C and D). In contrast, Nod2$^{-/-}$ mice were capable of producing HSA-specific Ig and IgG1 at a similar level to wild-type mice when they were immunized with resiquimod (R-848, a synthetic ligand for mouse TLR7) (Fig. 2, E and F) (*17*). These results indicate that Nod2 is able to activate adaptive immunity and to mediate adjuvant activity in the production of antibody to T cell–dependent antigens.

To investigate whether Nod2 plays a specific role in the innate immune response against bacterial infection, wild-type and Nod2$^{-/-}$ mice were challenged with the Gram-positive intracellular bacterium *Listeria monocytogenes* by intravenous injection. No significant difference was observed in the number of bacteria recovered from both the liver and the spleen 24 or 48 hours later (Fig. 3A). Similarly, serum IL-6 production, which normally accompanies bacterial infection, was not elevated. (Fig. 3B). Because *L. monocytogenes* naturally infects humans by the intestinal route, mice were also challenged with *L. monocytogenes* by intraperitoneal injection, and again no significant difference in survival between wild-type and Nod2$^{-/-}$ mice was seen (Fig. 3C). Consistent with these data, in vitro infection of macrophages from Nod2$^{-/-}$ mice with *L. monocytogenes* induced equivalent level of TNF-α and resulted in similar intracellular bacterial growth and killing (fig. S6, A to C). In contrast, Nod2$^{-/-}$ mice challenged with *L. monocytogenes* via intragastric dosing were susceptible to infection and showed signifi-

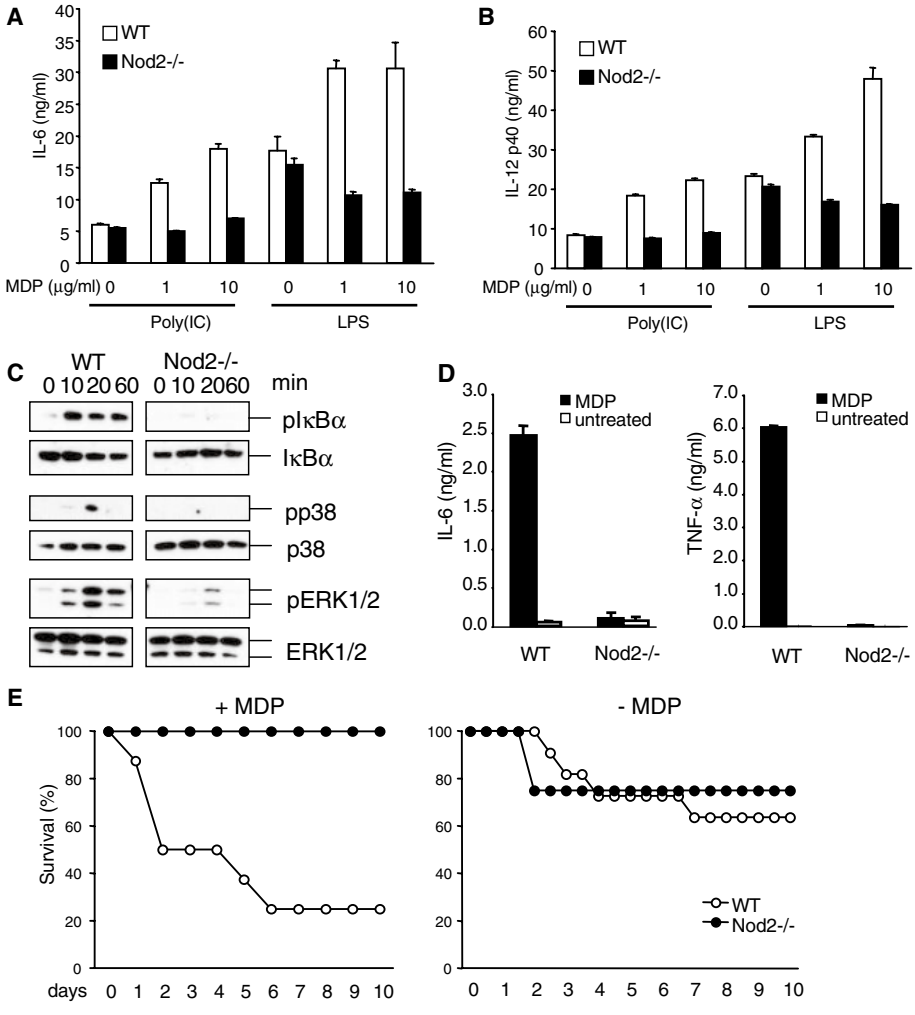

Fig. 1. Nod2 cooperates with TLR signaling and is required for MDP recognition in vivo. (**A**) BMDMs from wild-type (WT) and Nod2$^{-/-}$ mice were stimulated with LPS (10 ng/ml) (from Alexis) or poly(I:C) (100 μg/ml) in the presence of MDP (0, 1, or 10 μg/ml) for 24 hours. Production of IL-6 was assessed by ELISA. (**B**) Production of IL-12 p40 was assessed as in (A). (**C**) BMDMs from wild-type and Nod2$^{-/-}$ mice were stimulated with MDP (10 μg/ml) for indicated periods. Total cell lysates were prepared and blotted with antibodies against phosphorylated (p) and unphosphorylated forms of IκBα, p38, and ERK1/2. (**D**) Immature BMDCs were stimulated with MDP (25 μg/ml) for 20 hours, and production of IL-6 and TNF-α was assessed by ELISA. (**E**) Wild-type (n = 8) and Nod2$^{-/-}$ (n = 6) mice (8 to 12 weeks old) were primed with MDP (250 μg) and challenged with LPS (250 μg, Ultrapure LPS from InvivoGen). The survival of each mouse genotype was plotted. As a control, wild-type (n = 11) and Nod2$^{-/-}$ (n = 8) mice were challenged with LPS without MDP priming.

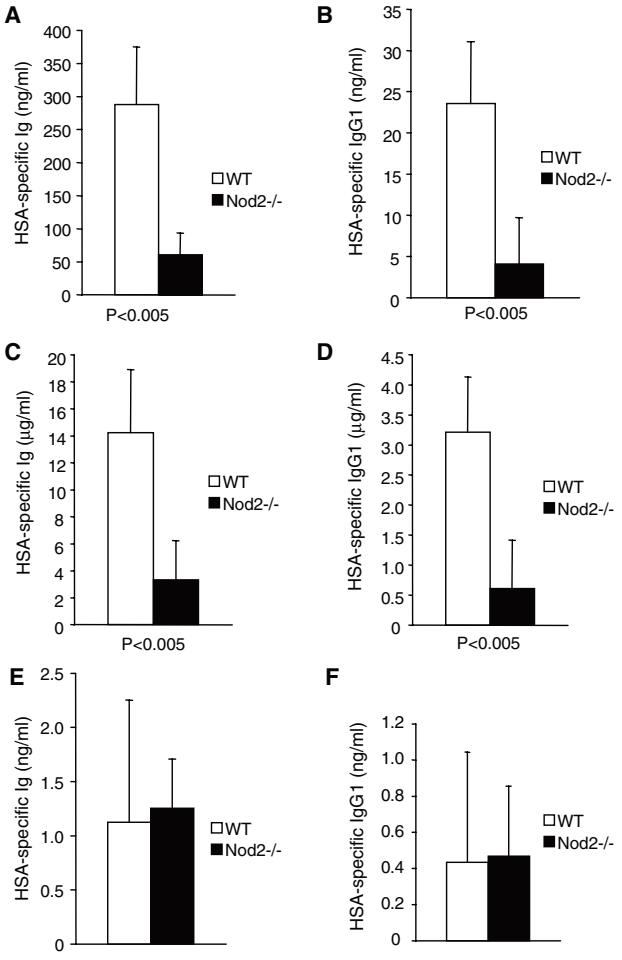

Fig. 2. Impaired antigen-specific immunoglobulin production in Nod2$^{-/-}$ mice immunized with MDP. (**A**) Wild-type (n = 5) and Nod2$^{-/-}$ (n = 4) mice (8 to 12 weeks old) were immunized with HSA (100 μg) and MDP (50 μg) and bled 2 weeks after immunization. Antigen-specific Ig was measured by ELISA. The P value was determined by Student's t-test. (**B**) Antigen-specific IgG1 was assessed by ELISA as in (A). (**C**) The immunized mice were boosted 3 weeks later with HSA (20 μg) and MDP (10 μg) and bled 1 week later. Antigen-specific Ig was measured by ELISA. (**D**) Antigen-specific IgG1 was assessed by ELISA as in (C). (**E**) Wild-type (n = 5) and Nod2$^{-/-}$ (n = 4) mice (8 to 12 weeks old) were immunized with HSA (100 μg) and R-848 (100 nmol) and bled 2 weeks after immunization. Antigen-specific Ig was measured as in (A). (**F**) Antigen-specific IgG1 was assessed by ELISA as in (E).

cantly greater numbers of bacteria recovered from both the liver and the spleen than did wild-type mice (Fig. 3D). These results suggest that Nod2 plays a pivotal and specific role in protecting against bacterial infection in the intestine. The number of *L. monocytogenes* in intestinal Peyer's patches was not significantly different between wild-type and Nod2$^{-/-}$ mice, which suggests that Nod2 might be involved in a Peyer's patch–independent route of bacterial invasion (fig. S7). Expression of Nod2 in the terminal ileum was examined by using purified villi and crypts (Fig. 3E) (*18*). Reverse transcription polymerase chain reaction (RT-PCR) revealed that Nod2 is highly expressed in crypts but not in villi, which is consistent with a recent report showing expression in human intestinal Paneth cells (Fig. 3F) (*19*, *20*).

To investigate potential genes that might be induced by Nod2 during intestinal infection, we isolated RNA samples from both the wild-type and Nod2$^{-/-}$ terminal ileum before and after *Listeria* infection by gastric gavage and screened them by microarray analysis (table S2). The up-regulation of candidate genes, which might explain the susceptibility of Nod2$^{-/-}$ mice to bacterial infection, was confirmed by quantitative real-time PCR (Fig. 3, G to I). The most significant difference was in the expression of a subgroup of cryptdins (called α-defensins in humans). Thus, expression of defensin-related cryptdin 4 (Defcr4) and

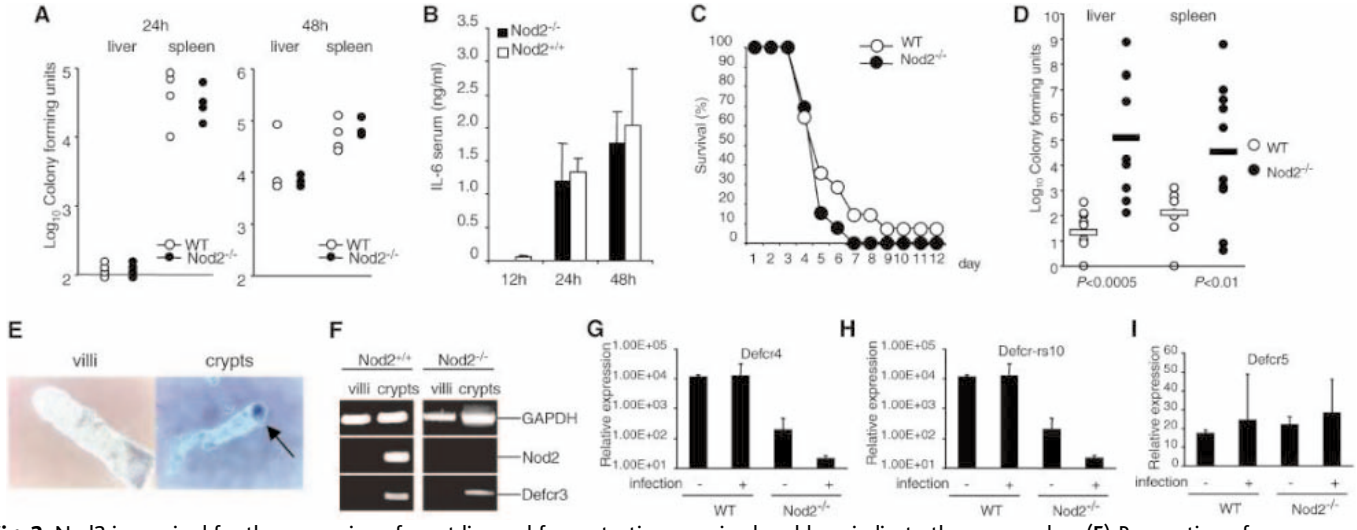

Fig. 3. Nod2 is required for the expression of cryptdins and for protection against intracellular bacterial infection in the intestine. (**A**) Wild-type and Nod2$^{-/-}$ mice (8 to 12 weeks old) were infected with 3 × 10^3 *L. monocytogenes* (strain 10403S) intravenously, and the number of bacteria in the liver and the spleen was counted 24 and 48 hours after infection. (**B**) Wild-type (n = 5) and Nod2$^{-/-}$ (n = 5) mice were infected with 10^4 *L. monocytogenes* intravenously and serum IL-6 was measured by ELISA. (**C**) Wild-type (n = 14) and Nod2$^{-/-}$ (n = 13) mice were infected with 10^6 *L. monocytogenes* intraperitoneally and the survival of the mice was plotted. (**D**) Wild-type (n = 10) and Nod2$^{-/-}$ (n = 10) mice were infected with 10^9 *L. monocytogenes* intragastrically by gastric gavage, and the number of bacteria in the liver and the spleen was counted 72 hours after infection. The P value was determined by Mann-Whitney test. Circles indicate each animal and bars indicate the mean value. (**E**) Preparation of mouse small intestinal crypts and villi. Individual mouse crypts were stained for 2 min with 0.25% amido black (marked by an arrow). (**F**) Villi and crypts were isolated from the terminal ileum of wild-type and Nod2$^{-/-}$ mice, and the expression of Nod2 was examined by RT-PCR. Glyceraldehyde phosphate dehydrogenase (GAPDH) and Defcr3 were used for loading control and quality of the crypt preparation, respectively. (**G** to **I**) Wild-type and Nod2$^{-/-}$ mice (8 to 12 weeks old) were infected with 10^9 *L. monocytogenes* intragastrically by gastric gavage. mRNA was extracted from the terminal ileum from Nod2$^{-/-}$ mice before (n = 5) and 24 hours after (n = 4) infection and from littermate wild-type mice before (n = 9) and 24 hours after (n = 4) infection. The expression of Defcr4, Defcr-rs10, and Defcr5 was examined by quantitative real-time PCR.

Defcr-related sequence 10 (Defcr-rs10) was very low in Nod2$^{-/-}$ mice and was further reduced after infection in Nod2$^{-/-}$ animals relative to wild-type mice (Fig. 3, G and H). Cryptdins are antimicrobial peptides that are preferentially produced in intestinal Paneth cells, and their antimicrobial activity is important in suppressing infection with pathogenic bacteria, including *L. monocytogenes* (*21*) and *Mycobacterium paratuberculosis*, an organism implicated in CD (*22*, *23*). Of the cryptdins, Defcr4 has the most potent bactericidal activity (*24*), with its expression being highest in the lower ileum, in contrast to other cryptdins (*25*, *26*). By comparison, Defcr5 was expressed normally in Nod2$^{-/-}$ mice both before and after infection (Fig. 3I) (*25*).

Our results indicate that Nod2 is essential in the detection of bacterial MDP and is capable of activating the adaptive immune system by acting as an adjuvant receptor for antibody production, either directly or by enhancing the production of α-defensins (*27*, *28*) or other immunostimulatory molecules. Therefore, Nod2 is critical in protecting the host from intestinal bacterial infection. More specifically, we reveal an important role for Nod2 in the regulation of a subgroup of cryptdins, offering a plausible mechanism to explain the association between Nod2 and susceptibility to CD. Murine cryptdins represent a more diverse family than those of human α-defensins and are already known to be critical in the innate immune responses to bacterial infection (*29*). CD-associated Nod2 mutations predispose primarily to ileal lesions (*30*–*33*), corresponding to the location of Paneth cells. Recent reports suggest that the expression of α-defensins is diminished in human CD patients, particularly those who have Nod2 gene mutations (*34*, *35*). However, it remains to be established whether a defect in Paneth cell function is the only possible mechanism by which Nod2 mutations might associate with the development of CD in humans. Nevertheless, it seems reasonable to suggest that mutations in Nod2 might promote CD through defective regulation of responses to commensal and/or pathogenic bacteria, rather than acting as an initiating factor for disease. Further studies may resolve this issue and may lead to the development of more rational therapeutic approaches for treating CD.

References and Notes

1. Y. Ogura *et al.*, *Nature* **411**, 603 (2001).
2. J. P. Hugot *et al.*, *Nature* **411**, 599 (2001).
3. N. Inohara *et al.*, *J. Biol. Chem.* **278**, 5509 (2003).
4. S. E. Girardin *et al.*, *J. Biol. Chem.* **278**, 8869 (2003).
5. J. Li *et al.*, *Hum. Mol. Genet.* **13**, 1715 (2004).
6. J. Hampe *et al.*, *Lancet* **357**, 1925 (2001).
7. Y. Ogura *et al.*, *J. Biol. Chem.* **276**, 4812 (2001).
8. A. Poltorak *et al.*, *Science* **282**, 2085 (1998).
9. S. T. Qureshi *et al.*, *J. Exp. Med.* **189**, 615 (1999).
10. K. Hoshino *et al.*, *J. Immunol.* **162**, 3749 (1999).
11. A. O. Aliprantis *et al.*, *Science* **285**, 736 (1999).
12. L. Alexopoulou, A. C. Holt, R. Medzhitov, R. A. Flavell, *Nature* **413**, 732 (2001).
13. O. Gutierrez *et al.*, *J. Biol. Chem.* **277**, 41701 (2002).
14. H. Takada, S. Yokoyama, S. Yang, *J. Endotoxin Res.* **8**, 337 (2002).
15. T. Watanabe, A. Kitani, P. J. Murray, W. Strober, *Nature Immunol.* **5**, 800 (2004).
16. M. Schnare *et al.*, *Nature Immunol.* **2**, 947 (2001).
17. H. Hemmi *et al.*, *Nature Immunol.* **3**, 196 (2002).
18. T. Ayabe *et al.*, *Nature Immunol.* **1**, 113 (2000).
19. S. Lala *et al.*, *Gastroenterology* **125**, 47 (2003).
20. Y. Ogura *et al.*, *Gut* **52**, 1591 (2003).
21. P. B. Eisenhauer, S. S. Harwig, R. I. Lehrer, *Infect. Immun.* **60**, 3556 (1992).
22. S. A. Naser, G. Ghobrial, C. Romero, J. F. Valentine, *Lancet* **364**, 1039 (2004).
23. W. S. Selby, *Lancet* **364**, 1013 (2004).
24. A. J. Ouellette *et al.*, *Infect. Immun.* **62**, 5040 (1994).
25. A. J. Ouellette *et al.*, *Infect. Immun.* **67**, 6643 (1999).
26. D. Darmoul, A. J. Ouellette, *Am. J. Physiol.* **271**, G68 (1996).
27. K. Tani *et al.*, *Int. Immunol.* **12**, 691 (2000).
28. D. Yang, A. Biragyn, L. W. Kwak, J. J. Oppenheim, *Trends Immunol.* **23**, 291 (2002).
29. C. L. Wilson *et al.*, *Science* **286**, 113 (1999).
30. T. Ahmad *et al.*, *Gastroenterology* **122**, 854 (2002).
31. A. P. Cuthbert *et al.*, *Gastroenterology* **122**, 867 (2002).
32. S. Lesage *et al.*, *Am. J. Hum. Genet.* **70**, 845 (2002).
33. J. Hampe *et al.*, *Lancet* **359**, 1661 (2002).
34. M. Schmid, K. Fellermann, J. Wehkamp, K. Herrlinger, E. F. Stange, *Z. Gastroenterol.* **42**, 333 (2004).
35. J. Wehkamp *et al.*, *Gut* **53**, 1658 (2004).

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Supporting Online Material

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Materials and Methods
Figs. S1 to S7
Tables S1 and S2
References

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Nod2 Mutation in Crohn's Disease Potentiates NF-κB Activity and IL-1β Processing

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Variants of NOD2, an intracellular sensor of bacteria-derived muramyl dipeptide (MDP), increase susceptibility to Crohn's disease (CD). These variants are thought to be defective in activation of nuclear factor κB (NF-κB) and antibacterial defenses, but CD clinical specimens display elevated NF-κB activity. To illuminate the pathophysiological function of NOD2, we introduced such a variant to the mouse *Nod2* locus. Mutant mice exhibited elevated NF-κB activation in response to MDP and more efficient processing and secretion of the cytokine interleukin-1β (IL-1β). These effects are linked to increased susceptibility to bacterial-induced intestinal inflammation and identify NOD2 as a positive regulator of NF-κB activation and IL-1β secretion.

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) thought to be caused by genetic and environmental factors that affect host-microbe interactions and production of inflammatory mediators (*1*, *2*). Mutations that increase susceptibility to CD up to 40 times were mapped to the *NOD2/CARD15* locus (*3*, *4*). The NOD2 protein contains two N-terminal caspase recruitment domains (CARDs), a nucleotide-binding domain (NBD), and 10 C-terminal leucine-rich repeats (LRRs), and it is expressed mainly by macrophages and dendritic cells (*5*). NOD2 mediates intracellular recognition of MDP, a building block for bacterial cell walls (*6*, *7*), and can activate NF-κB (*5*). Macrophages within the intestinal lamina propria of CD patients overproduce NF-κB targets, including the proinflammatory cytokines tumor necrosis factor–α (TNFα) and the interleukins IL-1β and IL-6 (*2*, *8*). Many of the anti-inflammatory drugs used to treat CD inhibit NF-κB activation, which suggests it is a key pathogenic factor (*8*, *9*). However, paradoxically, transient transfection experiments indicate that CD-associated

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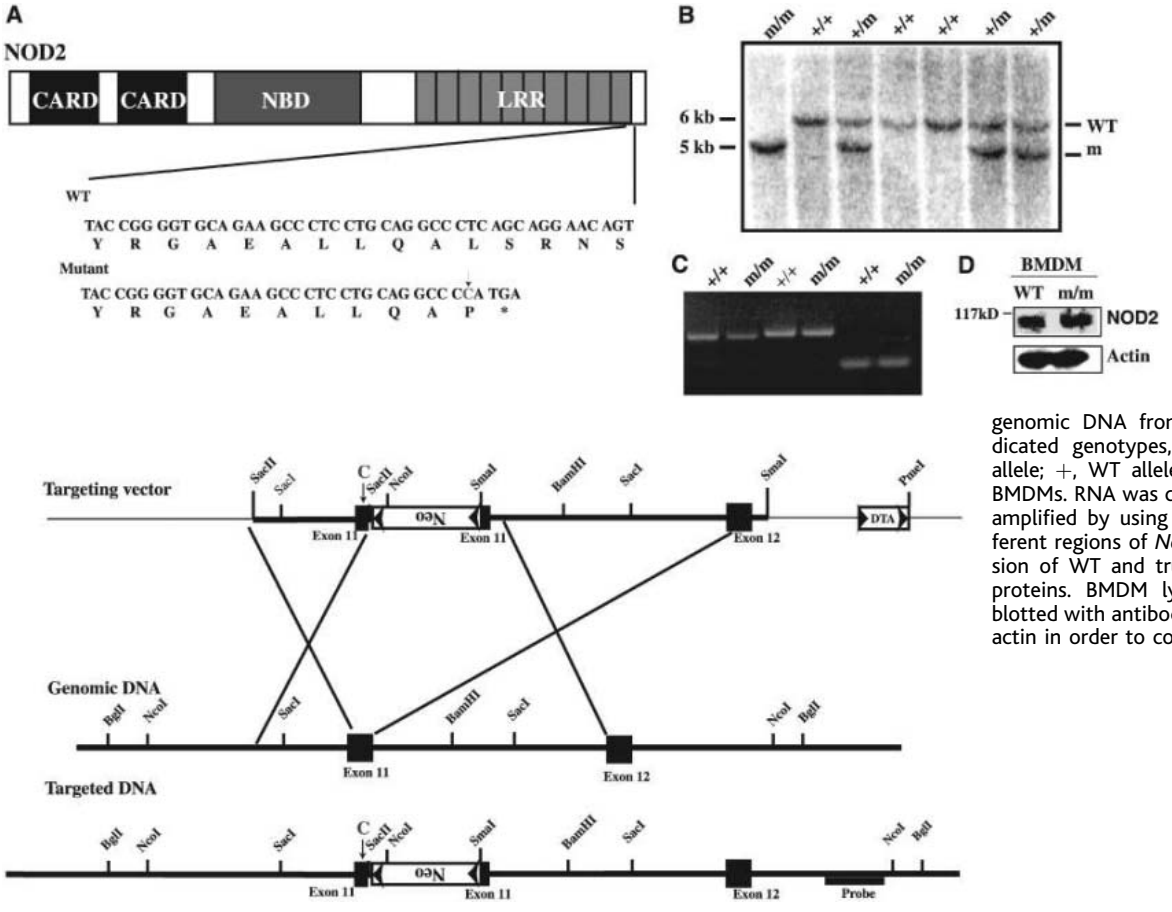

Fig. 1. Generation of *Nod2*^{2939iC} mice. (**A**) Schematic structure of NOD2, sequence of WT, and mutant alleles around the *2939insC* mutation, targeting vector, and the targeted locus. Solid boxes represent exons, and lines introns. The *Neo*^{r} cassette was inserted opposite the *Nod2* transcription unit. (**B**) Southern blot analysis of Nco I–digested genomic DNA from F_2 mice of the indicated genotypes, where m is mutant allele; +, WT allele. (**C**) *Nod2* mRNA in BMDMs. RNA was converted to cDNA and amplified by using primers for three different regions of *Nod2* cDNA. (**D**) Expression of WT and truncated (m/m) NOD2 proteins. BMDM lysates were immunoblotted with antibodies against NOD2 and actin in order to control loading.

NOD2 variants no longer activate NF-κB in response to MDP (*6*, *7*), which suggests that defective NF-κB activation in macrophages facilitates infection of the lamina propria by enteric bacteria. However, macrophages can activate NF-κB in response to bacteria independently of NOD2 (*10*), and *Nod2* gene ablation did not cause spontaneous intestinal infections or colonic inflammation (*11*).

To find an explanation for these quandaries and to illuminate the mechanism by which CD-associated *NOD2* variants act, we generated mice whose *Nod2* locus harbors the homolog of the most common CD susceptibility allele, *3020insC*, which encodes a truncated protein lacking the last 33 amino acids (*3*, *4*). This was done through insertion of cytosine at position 2939 (corresponding to 3020 in human *NOD2*) of the *Nod2* open reading frame (Fig. 1, A and B). Homozygous *Nod2*^{2939iC} mice were obtained at the expected mendelian ratio and did not show abnormalities of the gastrointestinal tract (fig. S1) or other organs; they were healthy (*12*). The mutation had no effect on Nod2 mRNA or protein amounts in bone marrow–derived macrophages (BMDMs) (Fig. 1, C and D).

We examined the effect of the *Nod2*^{2939ic} mutation on NF-κB activation in BMDM cultures. The activity of IKK, which is the inhibitor of κB (IκB) kinase, the degradation of IκBα, and NF-κB DNA binding activity were higher in MDP-stimulated *Nod2*^{2939iC} macrophages than in wild-type (WT) cells (Fig. 2A). Only marginal differences in mitogen-activated protein kinases (MAPKs) were observed (fig. S2). No genotype-specific differences in NF-κB activation were observed after macrophage treatment with other microbial components that activate Toll-like receptors (TLRs) (*10*), including the TLR2 agonists Pam_3Cys (tripalmitoyl-*S*-glyceryl-Cys-Ser-4(Lys)) and peptidoglycan (PGN), the TLR4 agonist lipopolysaccharide (LPS), and the TLR9 agonist nonmethylated CpG-containing DNA (Fig. 2B) (*12*). Expression of several NF-κB target genes was increased in MDP-treated *Nod2*^{2939iC} macrophages relative to WT counterparts (Fig. 2C). Only minor differences in expression of these genes were observed when macrophages were stimulated with LPS or PGN. Although MDP-induced gene expression of several cytokine genes was increased in *Nod2*^{2939iC} macrophages, only IL-1β secretion was significantly elevated in these cells relative to WT counterparts (Fig. 2, D and E, fig. S3). Secretion of IL-1α was modestly elevated, and neither IL-6 nor TNFα were secreted in response to MDP. The only microbial product that stimulated IL-1β secretion by *Nod2*^{2939iC} macrophages was MDP (Fig. 2E).

Macrophages involved in CD most likely reside in the lamina propria (*2*). To expose these cells to enteric bacteria, mice were treated with dextran sodium sulfate (DSS), an agent that kills mucosal epithelial cells and disrupts their barrier function, causing bacterial invasion (*13*). WT and homozygous *Nod2*^{2939iC} mice (8 to 12 weeks old) were given 3% DSS in drinking water for 6 days and monitored for weight loss, a characteristic of severe intestinal inflammation. After 8 days, body weight loss was greater in *Nod2*^{2939iC} mice relative to WT mice (Fig. 3A). *Nod2*^{2939iC} mice also exhibited increased mortality relative to WT mice (37.5% versus 0%) (fig. S4). Surviving mice of both genotypes regained body weight after day 11 and returned to normal 30 days after DSS administration (*12*). Histological analyses revealed that the severity and extent of inflammatory lesions in the colons of *Nod2*^{2939iC} mice were significantly ($P < 0.05$) greater than in WT controls, with larger areas of ulceration and increased infiltration of F4/80-positive macrophages (Fig. 3B, fig. S5).

After DSS exposure, *Nod2*^{2939iC} homozygotes expressed greater amounts of mRNAs encoding proinflammatory cytokines and che-

mokines in their colons relative to WT mice (Fig. 3C). The amounts of IL-1β, IL-6, and cyclooxygenase-2 (Cox-2) protein were significantly higher in colons of DSS-treated *Nod2*^{2939iC} mice relative to WT counterparts (Fig. 3D). IL-6 and Cox-2 were predominantly expressed in F4/80-positive macrophages within inflammatory lesions (Fig. 3E, fig. S6) (*12*). IKK and NF-κB activities and RelA(p65) nuclear staining were also higher in colons of *Nod2*^{2939iC} mice than in the WT (Fig. 3F, fig. S7). MAPK activation, however, was only marginally affected by the genotype (fig. S8).

The intestinal inflammatory response to DSS is dramatically reduced by oral antibiotics, which supports involvement of enteric bacteria (*14*). When given a high dose of DSS (6%) without oral antibiotics, WT and *Nod2*^{2939iC} mice died within 9 days after DSS administration (*12*), but mice that received oral antibiotics survived and developed mild inflammation and weight loss, without any genotype-linked differences (fig. S9). Thus, enteric bacteria elicit the inflammatory response to DSS, and without bacterial exposure, *Nod2*^{2939iC} mice have the same reaction as WT counterparts.

Exposure of macrophages to bacteria activates inflammatory and apoptotic caspases (*15*). More apoptotic cells, most of which were positive for the F4/80 macrophage marker, were found in the lamina propria of DSS-treated *Nod2*^{2939iC} mice than in WT counterparts (Fig. 4, A and B). Increased macrophage apoptosis is associated with activation of caspase-1 (*16*), an enzyme required for secretion of mature IL-1β (*17*, *18*). Congruently, only background levels of secreted IL-1β were present in colons of untreated mice, but IL-1β concentrations were elevated after DSS treatment, particularly in *Nod2*^{2939iC} mice (Fig. 3D). Macrophage activation with LPS induces pro–IL-1β, but its processing and release require activation of caspase-1 by a different signal (*16*). LPS did not induce secretion of mature IL-1β in either *Nod2*^{2939iC} or WT macrophages, although it stimulated TNFα release (Fig. 2, D and E). In contrast, MDP stimulated release of mature IL-1β but not TNFα by *Nod2*^{2939iC} macrophages. To determine whether IL-1β secretion may be involved in the increased inflammatory response to DSS in *Nod2*^{2939iC} mice, mice were injected once daily with IL-1 receptor antagonist (IL-1-RA) from the start of DSS exposure. Average body weight loss and histological score were improved in IL-1-RA–treated mice, and differences in weight loss (Fig. 4C) and inflammatory score (Fig. 4D, fig. S10) between the genotypes were abolished.

By contrast to the *Nod2*^{2939iC} mutation, deletion of *Ikkβ* in hematopoietic and myeloid cells reduced the inflammatory response to DSS (fig. S11). However, its deletion in enterocytes increased the inflammatory response to DSS (*19*).

Collectively, our results suggest that *Nod2*^{2939iC} is a gain-of-function allele, whose product induces elevated IKK and caspase-1 activation in response to MDP. Although NOD2 was suggested to be a negative regulator of TLR2 (*20*), we found no effect of the *Nod2*^{2939iC} mutation on signaling by TLR2,

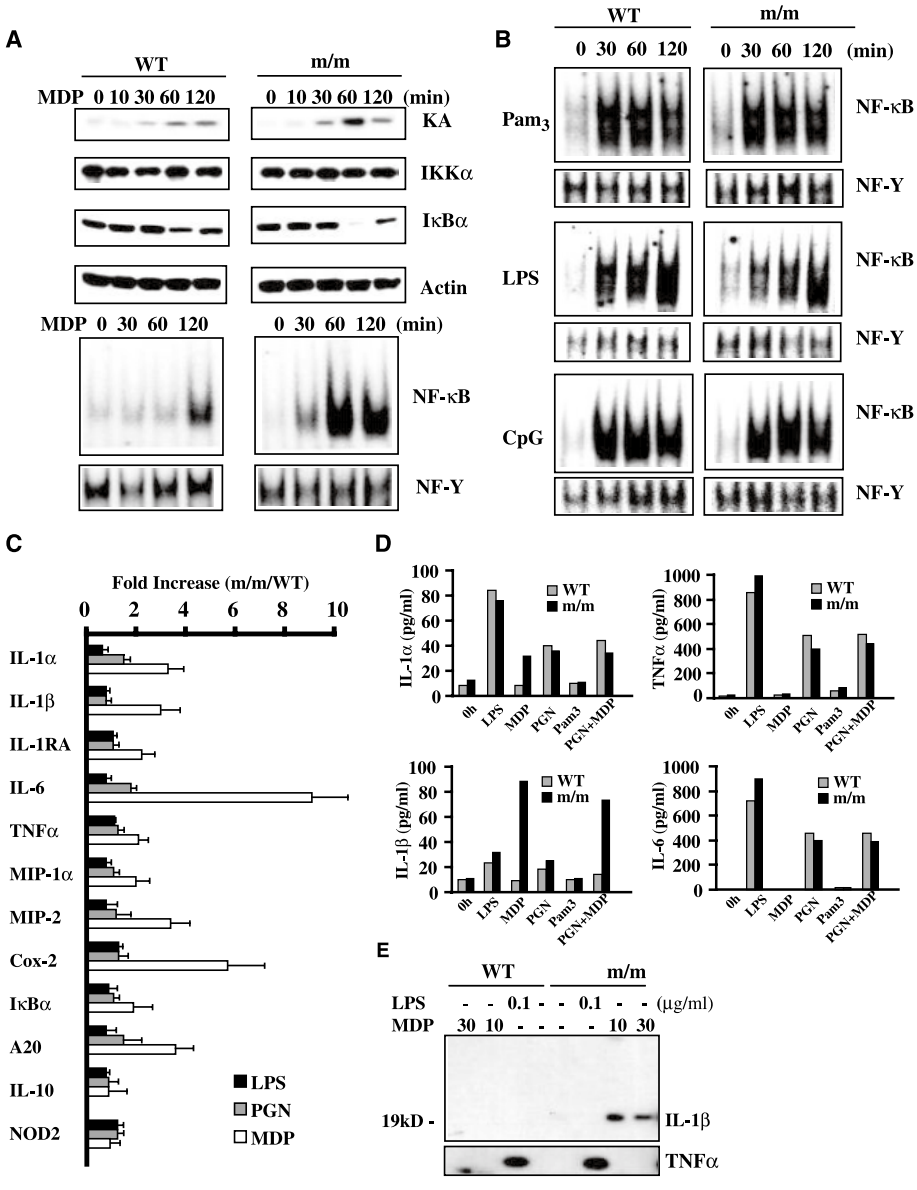

Fig. 2. *Nod2*^{2939iC} macrophages exhibit elevated NF-κB activation and IL-1β secretion in response to MDP. (**A**) BMDMs from WT and *Nod2*^{2939iC} (m/m) mice were incubated with MDP (1 μg/ml). Where indicated, cytosolic and nuclear extracts were prepared and used to analyze IKK activation (KA), IκBα degradation, and NF-κB DNA binding activity, respectively. Nuclear extract quality was monitored by measuring nuclear factor-Y (NF-Y) DNA binding. (**B**) BMDMs were stimulated with Pam_3Cys (1 μg/ml), LPS (100 ng/ml), or CpG DNA (1 μM) to activate TLR2, 4, and 9, respectively. Where indicated, nuclear extracts were prepared and NF-κB DNA binding activity was analyzed. (**C**) Expression of NF-κB target genes was examined in *Nod2*^{2939iC} and WT macrophages stimulated with MDP, LPS, or peptidoglycan (PGN from *Staphylococcus aureus*, 10 μg/ml). After 4 hours, cells were collected, total cytoplasmic RNA was prepared, and gene expression was analyzed by real-time polymerase chain reaction (PCR). Data are presented as the fold increase in mRNA expression in *Nod2*^{2939iC} macrophages relative to WT macrophages, which was given an arbitrary level of 1.0 for each gene. Results are means ± SEM of three independent experiments. (**D**) Elevated IL-1β secretion in MDP-stimulated *Nod2*^{2939iC} macrophages. WT and *Nod2*^{2939iC} (m/m) BMDMs were stimulated as indicated. After 24 hours, culture supernatants were collected and secreted cytokines were measured. (**E**) MDP induces IL-1β release by *Nod2*^{2939iC} (m/m) BMDMs. Macrophages were treated with MDP or LPS for 24 hours. Culture supernatants were collected and analyzed by immunoblotting with antibodies against IL-1β and TNFα.

as coincubation of macrophages with MDP plus a TLR2 agonist (PGN) did not reduce the response to PGN (Fig. 2D). The inhibitory function hypothesis is also inconsistent with in vivo findings in *Nod2* knockout mice, which did not show increased inflammation (*11*). The gain-of-function hypothesis is consistent with clinical observations made in CD patients (*8*, *21*).

The NF-κB signaling pathway induces many proinflammatory genes coding for cytokines and chemokines, including IL-1β, TNFα, and IL-6 (*22*, *23*), and may therefore be an important pathogenic factor in CD (*8*). Although increased transcription of many

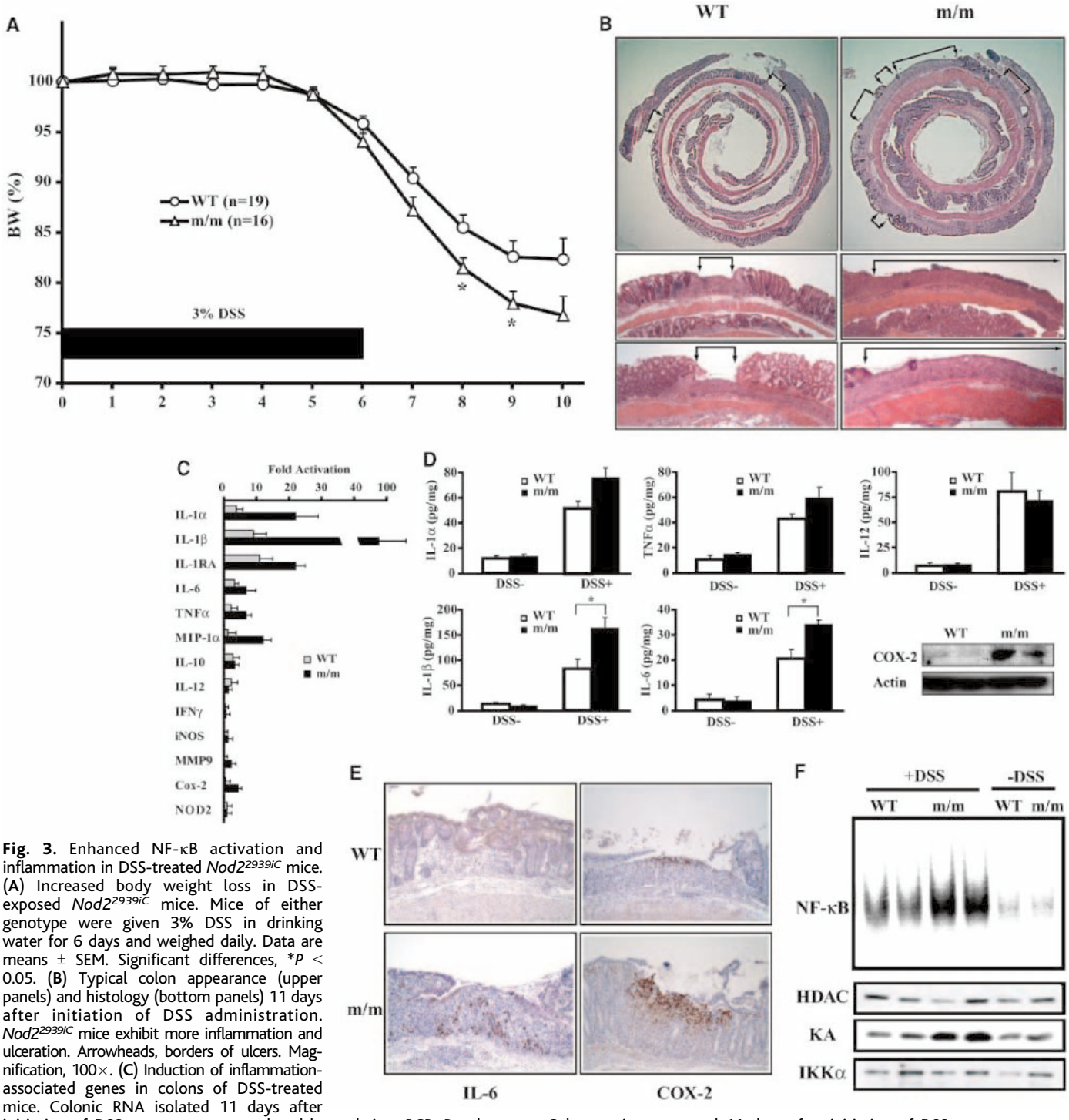

Fig. 3. Enhanced NF-κB activation and inflammation in DSS-treated *Nod2*^{2939iC} mice. (**A**) Increased body weight loss in DSS-exposed *Nod2*^{2939iC} mice. Mice of either genotype were given 3% DSS in drinking water for 6 days and weighed daily. Data are means ± SEM. Significant differences, $*P < 0.05$. (**B**) Typical colon appearance (upper panels) and histology (bottom panels) 11 days after initiation of DSS administration. *Nod2*^{2939iC} mice exhibit more inflammation and ulceration. Arrowheads, borders of ulcers. Magnification, 100×. (**C**) Induction of inflammation-associated genes in colons of DSS-treated mice. Colonic RNA isolated 11 days after initiation of DSS treatment was analyzed by real-time PCR. Results are means ± SEM of fold increase in normalized (relative to glyceraldehyde-3-phosphate dehydrogenase mRNA) mRNA amounts in DSS-treated mice over untreated mice of the same genotype ($n = 4$ per group). (**D**) Elevated IL-1β and IL-6 in colons of DSS-treated *Nod2*^{2939iC} mice. The indicated cytokines were measured in colonic extracts prepared 0 or 11 days after DSS exposure. Results are means ± SD ($n = 4$ to 8). Significant difference, $*P < 0.05$. (**E**) Immunohistochemical detection of IL-6 and Cox-2. Colon sections prepared 11 days after initiation of DSS treatment were analyzed by indirect immunoperoxidase staining for IL-6 and Cox-2. Magnification, 100×. (**F**) Colonic NF-κB and IKK activities. Nuclear and cytosolic extracts of colonic mucosa prepared 0 and 11 days after initiation of DSS administration were analyzed for NF-κB DNA binding and IKK kinase (KA) activities. Protein recovery in nuclear extracts was determined by immunoblotting with antibody against histone deacetylase (HDAC), and IK recovery was determined with antibody against IKK (IKKα).

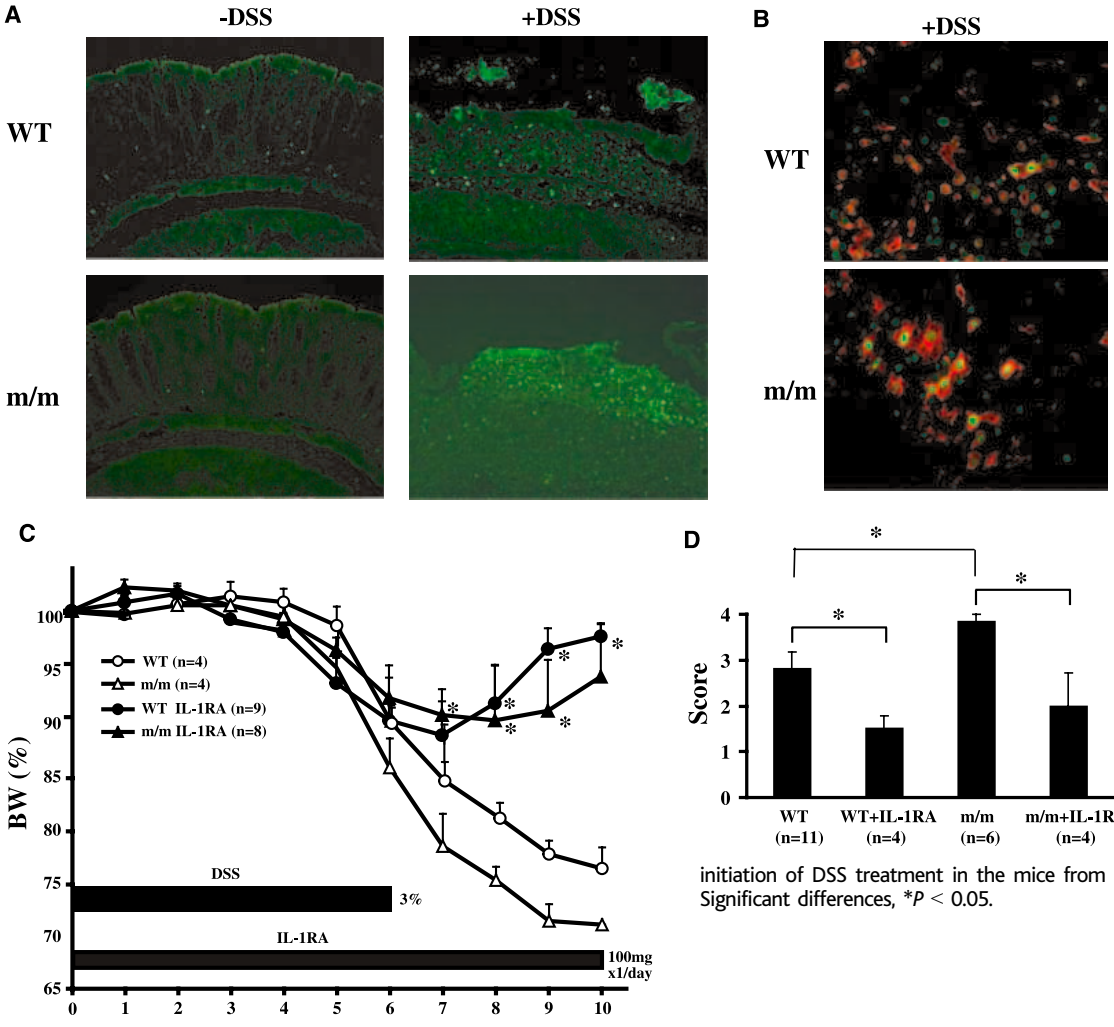

Fig. 4. IL-1β is an important contributor to elevated colonic inflammation in *Nod2*^{2939iC} mice. (**A** and **B**) Increased macrophage apoptosis in *Nod2*^{2939iC} (m/m) mice treated with DSS. Tissue specimens prepared 0 and 11 days after initiation of DSS administration were analyzed by TUNEL staining (A) or by TUNEL (green) plus immunoperoxidase staining for F4/80 (red) (B). Magnification: (A), 200×; (B), 400×. (**C**) Increased body weight loss in DSS-exposed *Nod2*^{2939iC} mice is IL-1β dependent. Mice of either genotype were given 3% DSS for 6 days with or without concomitant treatment with IL-1RA (100 mg/kg per day). Mice were weighed daily. Data are means ± SEM. Asterisks indicate significant differences (WT versus WT IL-1-RA, m/m versus m/m IL-1-RA; $P < 0.05$). (**D**) Histological inflammation and tissue damage scores were determined 11 days after initiation of DSS treatment in the mice from (C). Results are means ± SEM. Significant differences, *$P < 0.05$.

NF-κB targets was observed, the results with IL-1β were unique, as it was the only pro-inflammatory cytokine whose secretion in response to MDP was markedly elevated in *Nod2*^{2939iC} macrophages relative to WT counterparts. Our results suggest that IL-1β is indeed an important contributor to the increased colonic inflammation in *Nod2*^{2939iC} mice, as previously suggested for CD patients (*2*).

Although NF-κB was thought to be the major effector for NOD2, it should be noted that NF-κB is more effectively activated by bacterial products through TLRs (see Fig. 2). Thus NF-κB activation is not unique to NOD2, and its loss may not compromise NF-κB signaling in response to bacterial infection. Recently, TLR signaling and a certain amount of enteric bacteria were shown to be critical for maintenance of the intestinal barrier function (*24*), a function that was suggested to deteriorate in CD patients (*2*). However, maintenance of barrier function is unlikely to involve NOD2. By contrast, a unique function of NOD2, not provided by TLRs, is induction of IL-1β processing and release. This function may be mediated through the N-terminal CARD domains of NOD2, which may directly interact with caspase-1 or upstream caspases. Given the importance of IL-1β for the pathology of DSS-induced colitis in *Nod2*^{2939iC} mice and the imbalance between IL-1β and IL-1-RA in CD patients (*2*), it would be of interest to critically evaluate its role in CD pathogenesis.

References and Notes

1. S. E. Girardin, J. P. Hugot, P. J. Sansonetti, *Trends Immunol.* **24**, 652 (2003).
2. C. Fiocchi, *Gastroenterology* **115**, 182 (1998).
3. Y. Ogura *et al.*, *Nature* **411**, 603 (2001).
4. J. P. Hugot *et al.*, *Nature* **411**, 599 (2001).
5. Y. Ogura *et al.*, *J. Biol. Chem.* **276**, 4812 (2001).
6. N. Inohara *et al.*, *J. Biol. Chem.* **278**, 5509 (2003).
7. S. E. Girardin *et al.*, *J. Biol. Chem.* **278**, 8869 (2003).
8. D. K. Podolsky, *N. Engl. J. Med.* **347**, 417 (2002).
9. C. Wahl, S. Liptay, G. Adler, R. M. Schmid, *J. Clin. Invest.* **101**, 1163 (1998).
10. E. Kopp, R. Medzhitov, *Curr. Opin. Immunol.* **15**, 396 (2003).
11. A. L. Pauleau, P. J. Murray, *Mol. Cell. Biol.* **23**, 7531 (2003).
12. S. Maeda *et al.*, unpublished observation.
13. W. Strober, I. J. Fuss, R. S. Blumberg, *Annu. Rev. Immunol.* **20**, 495 (2002).
14. H. C. Rath *et al.*, *Infect. Immun.* **69**, 2277 (2001).
15. Y. Weinrauch, A. Zychlinsky, *Annu. Rev. Microbiol.* **53**, 155 (1999).
16. R. A. Le Feuvre, D. Brough, Y. Iwakura, K. Takeda, N. J. Rothwell, *J. Biol. Chem.* **277**, 3210 (2002).
17. N. A. Thornberry, Y. Lazebnik, *Science* **281**, 1312 (1998).
18. F. Martinon, J. Tschopp, *Cell* **117**, 561 (2004).
19. F. R. Greten *et al.*, *Cell* **118**, 285 (2004).
20. T. Watanabe, A. Kitani, P. J. Murray, W. Strober, *Nature Immunol.* **5**, 800 (2004).
21. B. E. Sands *et al.*, *N. Engl. J. Med.* **350**, 876 (2004).
22. P. J. Barnes, M. Karin, *N. Engl. J. Med.* **336**, 1066 (1997).
23. L.-W. Chen *et al.*, *Nature Med.* **9**, 575 (2003).
24. S. Rakoff-Nahoum, J. Paglino, F. Eslami-Varzaneh, S. Edberg, R. Medzhitov, *Cell* **118**, 229 (2004).

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The Kaposin B Protein of KSHV Activates the p38/MK2 Pathway and Stabilizes Cytokine mRNAs

Craig McCormick and Don Ganem*

Cytokine production plays a critical role in diseases caused by Kaposi's sarcoma–associated herpesvirus (KSHV). Here we show that a latent KSHV gene product, kaposin B, increases the expression of cytokines by blocking the degradation of their messenger RNAs (mRNAs). Cytokine transcripts are normally unstable because they contain AU-rich elements (AREs) in their 3′ noncoding regions that target them for degradation. Kaposin B reverses this instability by binding to and activating the kinase MK2, a target of the p38 mitogen-activated protein kinase signaling pathway and a known inhibitor of ARE-mRNA decay. These findings define an important mechanism linking latent KSHV infection to cytokine production, and also illustrate a distinctive mode by which viruses can selectively modulate mRNA turnover.

Kaposi's sarcoma–associated herpesvirus (KSHV) is a γ-2 herpesvirus etiologically linked to Kaposi's sarcoma (KS) (*1*), an unusual tumor composed of proliferating, spindle-shaped endothelial cells, slit-like neovascular spaces, and inflammatory cell infiltration (*2*). KS spindle cells, which are latently infected with KSHV, elaborate a variety of pathogenetically important proinflammatory cytokines and angiogenic factors (*3*, *4*); in turn, cultured spindle cells require cytokines [including interleukin-6 (IL-6)] for their survival and proliferation (*5*). KSHV also causes several rare lymphoproliferative diseases (*6*, *7*), and these too are associated with elevated production of cytokines, notably IL-6 (*6*–*10*). However, the mechanisms linking KSHV and host cytokine production have remained obscure.

Cells latently infected by KSHV express only a handful of viral genes, including the products of the kaposin locus (*11*) (Fig. 1A). This locus contains a small coding region [open reading frame (ORF) K12] preceded by two families of 23-nucleotide GC-rich direct repeats (termed DR1 and DR2), and is transcribed as a single mRNA encompassing all three components. A complex translational program generates a variety of proteins from this mRNA (*12*). Kaposin B, the subject of this report, results from translation of the repeats alone and consists of a series of tandemly repeated copies of 23–amino acid peptides derived from translation of the DR2 (HPRNPARRTPGTRRGAPQEPGAA) and DR1 (PGTWCPPPREPGALLPGNLVPSS) repeats (*12*, *13*). Its function has heretofore been unknown.

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To identify proteins that interact with kaposin B, we used the DR2 and DR1 repeats to screen a library of infected cell cDNAs in the yeast two-hybrid system (*14*). This yielded a clone consisting of amino acids 188 to 400 of MK2 [mitogen-activated protein kinase (MAPK)–associated protein kinase 2], a protein kinase whose activity is controlled by phosphorylation by p38, a MAPK activated by inflammatory and other signals (*15*). Immunoprecipitation of FLAG epitope–tagged kaposin B results in efficient coprecipitation of cotransfected MK2 in 293T cells, confirming the interaction in vivo (Fig. 1B, bottom panel, lane 4). This interaction is direct: A purified, *Escherichia coli*–derived fusion protein of glutathione *S*-transferase (GST) and kaposin B, but not GST alone, efficiently binds to purified recombinant MK2 (Fig. 1C). Parallel experiments with GST-DR1 and GST-DR2 proteins mapped the interaction domain to the DR2 region (Fig. 1C). Using truncated MK2 proteins, we also mapped the domains of MK2 required for interaction with kaposin B. Kaposin B binds to a region in the C-lobe of the kinase domain of MK2, with residues 200 to 270 apparently critical for the interaction (Fig. 1D) (fig. S1). This region contains a highly conserved activation segment, including a site for phosphorylation by p38 MAPK (Thr^{222}) and a binding site for the autoinhibitory C-terminal domain of MK2 (*16*, *17*).

These in vitro binding findings are further corroborated by studies that examine the colocalization of the two proteins under various conditions in vivo. Expression of kaposin B in cells reveals that the protein is colocalized with MK2 in the nucleus (Fig. 1E), despite the absence of a classical nuclear localization signal in kaposin B. MK2 is known to be exported from the nucleus in response to inflammatory signals that activate the p38 MAPK pathway [e.g., tumor necrosis factor–α (TNF-α) and lipopolysaccharide (LPS)] (*18*). It is noteworthy that treatment of cells with LPS (Fig. 1E) or TNF (fig. S2) causes kaposin B and MK2 to be coexported from nucleus to cytosol.

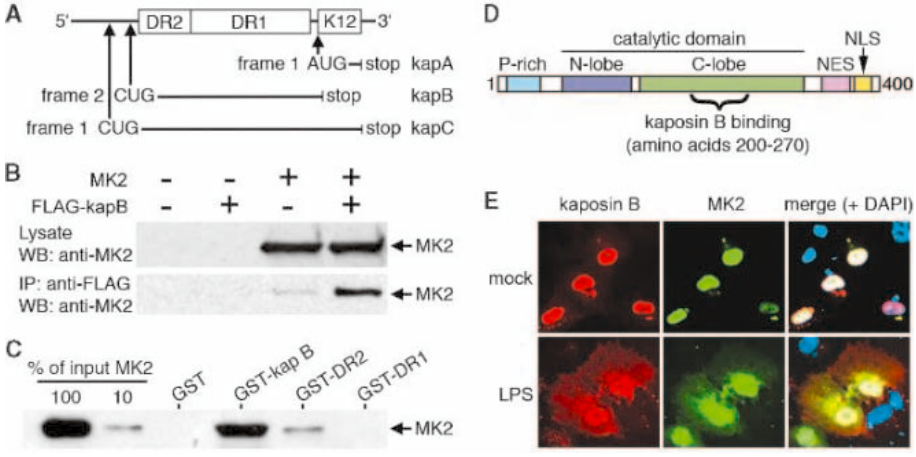

Fig. 1. (**A**) Schematic map of the K12 (kaposin) locus derived from a pulmonary KS isolate of KSHV (*12*). (**B**) Coimmunoprecipitation of kaposin B and MK2. 293T cells were cotransfected with expression vectors encoding MK2 and FLAG-tagged kaposin B. Cell lysates were immunoblotted with polyclonal antibody to MK2 (anti-MK2) (upper panel). In parallel, portions of the same lysates were immunoprecipitated with FLAG monoclonal antibody (mAb) and immunoblotted with anti-MK2 (lower panel). (**C**) Recombinant full-length MK2 purified from *E. coli* was incubated with the indicated GST fusion proteins bound to glutathione-Sepharose beads. MK2 was detected by Western blotting with anti-MK2. (**D**) Kaposin B binds to a central portion of the C-lobe of MK2. Truncated versions of MK2 were labeled with [^{35}S]methionine by in vitro translation and tested for binding to GST–kaposin B (see fig. S1 for details). NLS, nuclear localization signal; NES, nuclear export signal. (**E**) Coexport of kaposin B and MK2 in response to stress signals. SLK endothelial cells were cotransfected with plasmids encoding kaposin B and green fluorescent protein–MK2 fusion protein. After 30 hours, cells were treated with LPS (1 μg/ml) for 30 min, then fixed and stained with kaposin B mAb and rhodamine-conjugated secondary antibody. DAPI, 4′,6′-diamidino-2-phenylindole.

Binding of kaposin B to MK2 leads to activation of MK2, by several criteria. First, cells expressing kaposin B displayed higher levels of phosphorylated (activated) MK2 as judged by immunoblotting (Fig. 2A). Second, relative to MK2 precipitated from control cells, endogenous MK2 immunoprecipitated from cells expressing kaposin B was more active in phosphorylating the MK2 substrate heat shock protein 27 (Hsp27) (Fig. 2B).

Elevated MK2 kinase activity has a number of functional consequences, the best characterized of which is the stabilization of cytokine transcripts and other mRNAs that contain AU-rich elements (AREs). AREs typically consist of multiple copies of the sequence AUUUA, usually located in the 3′ untranslated region (3′UTR) of the transcript. Their presence leads to drastic destabilization of the mRNA in the ground state (*19*–*21*), but this instability can be abrogated by activation of MK2 (*22*). Accordingly, we tested the effects of kaposin B expression on this activity with the use of a standard assay of ARE-mediated mRNA decay (*23*) based on a globin reporter gene. Fig. 2C shows that the β-globin mRNA reporter is quite stable and is unaffected by cotransfection of kaposin B. However, the insertion of an ARE [here, from the 3′UTR of granulocyte-macrophage colony-stimulating factor (GM-CSF)] into the β-globin gene renders the chimeric mRNA susceptible to rapid degradation, and cotransfection of kaposin B with the β-globin–ARE vector strikingly reverses this effect. The magnitude of this stabilization is similar to that induced by other, more potent, triggers of the p38-MK2 pathway, including oxidative stress (Fig. 2D). Correspondingly, transfection of kaposin B into cells resulted in major augmentation of both GM-CSF and IL-6 production, as determined by enzyme-linked immunosorbent assay (ELISA) of the culture medium 48 hours after transfection (Fig. 2E) (fig. S3).

To determine whether ARE-mediated mRNA decay is also impaired in KSHV latency, we transfected HeLa–Tet Off cells with a β-globin–ARE expression vector, then infected the cells with KSHV virions; this resulted in a latent KSHV infection. Doxycycline was then added to arrest transcription, and β-globin mRNA levels were determined at 0, 1, 2, and 4 hours thereafter. Latently infected cells displayed substantial prolongation of the half-life of the ARE-containing transcript (Fig. 3, A and B). To establish that this effect was due to kaposin B and not to other latency proteins, we tested the known latent proteins for their ability to block ARE-mediated mRNA decay in transfected HeLa–Tet Off cells. None of the other known latency genes—those encoding latency-associated nuclear antigen (LANA) (*orf73*), v–cyclin D (*orf72*), v-FLIP (*orf71*), kaposin A (*K12*), or interferon regulatory factor v-IRF3—were active in ARE-dependent mRNA stabilization (Fig. 3C).

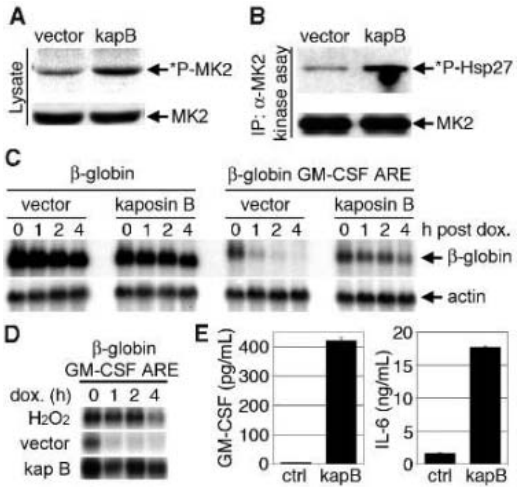

Fig. 2. Kaposin B stimulates MK2 kinase activity and blocks the degradation of cytokine mRNAs. (**A**) HeLa cells were transfected with empty vector or kaposin B expression vector, lysed, and harvested. Portions of the whole-cell lysates were immunoblotted with antibodies to MK2 (lower panel) and phosphorylated MK2 (upper panel). (**B**) Portions of the same HeLa cell lysates were immunoprecipitated with anti-MK2, and kinase activity was measured in vitro with recombinant Hsp27 as a substrate. (**C**) HeLa–Tet Off cells were cotransfected with β-globin–based reporter and test plasmids. After 30 hours, doxycycline was added to the media to stop transcription. RNA was harvested at 0, 1, 2, and 4 hours after doxycycline addition; β-globin mRNA was detected with a ^{32}P-labeled antisense riboprobe. (**D**) HeLa–Tet Off cells were transfected with a β-globin–ARE expression vector and either an empty vector or kaposin B expression vector. One hour before addition of doxycycline, 1 mM hydrogen peroxide was added to one vector-transfected culture (top panel). Samples of RNA were Northern blotted for β-globin mRNA as above. (**E**) Human foreskin fibroblasts were transfected with empty vector or kaposin B expression vector. After 48 hours, cell supernatants were collected and extracellular GM-CSF and IL-6 levels were measured by ELISA.

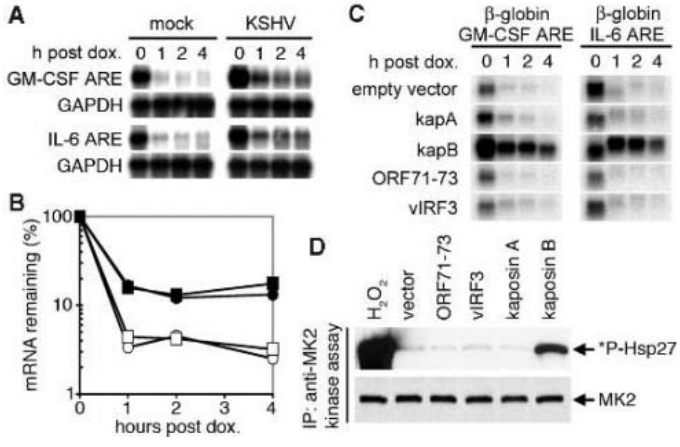

Fig. 3. Latent KSHV infection blocks degradation of ARE-containing mRNAs. (**A**) HeLa–Tet Off cells were transfected with β-globin–ARE reporter 24 hours before infection with KSHV. At 24 hours after infection, doxycycline was added to the media to stop transcription. RNA was harvested at 0, 1, 2, and 4 hours after doxycycline addition; β-globin mRNA was detected with a ^{32}P-labeled antisense riboprobe. (**B**) Phosphorimager-based quantitation of the results. Open circles, GM-CSF ARE + vector; solid circles, GM-CSF ARE + kaposin B; open squares, IL-6 ARE + vector; solid squares, IL-6 ARE + kaposin B. (**C**) Other KSHV latency genes do not block ARE-mediated mRNA decay. HeLa–Tet Off cells were cotransfected with the indicated β-globin–ARE reporter and test plasmid. After 30 hours, doxycycline was added and RNA was harvested and Northern blotted as above. (**D**) HeLa cells were transfected with plasmids encoding kaposin B or other latency-associated KSHV genes. At 30 hours after transfection, cells were lysed and immunoprecipitated with anti-MK2. MK2 kinase activity was assayed with Hsp27 substrate and an antibody to phosphorylated Hsp27 (top panel). Total immunoprecipitated MK2 was measured with anti-MK2 (bottom panel).

Correspondingly, the only latent gene product capable of activating MK2 kinase activity in such transfectants was kaposin B (Fig. 3D).

How does kaposin B expression lead to MK2 activation? Although our understanding of this process is still incomplete, important clues emerged when we examined the state of activation of the other components of the p38 MAPK pathway. HeLa cells transfected with kaposin B display activation of p38 activity as well as that of MK2 (Fig. 4A). In fact, both p38 and MKK6, an upstream kinase implicated in p38 activation, are coimmunoprecipitated from cell lysates along with kaposin B–MK2 complexes (Fig. 4B). Although MK2 binds very efficiently to kaposin B (Figs. 1C and 4C), to date we have detected only very weak binding of purified p38 to recombinant kaposin B in vitro (Fig. 4C). This suggests that p38 is recruited to kaposin-MK2 complexes principally (though perhaps not exclusively) via its binding to MK2. The enhanced p38 activity observed in kaposin B–transfected cells is an important contributor to the net state of MK2 activation in such cells, because blockade of p38 activity with the selective inhibitor SB203580 results in substantial (although not complete) reduction of MK2 activation (Fig. 4D) and cytokine release (fig. S4).

These results are consistent with a model (Fig. 4E) in which initial MK2 activation after its binding by kaposin B is amplified by p38

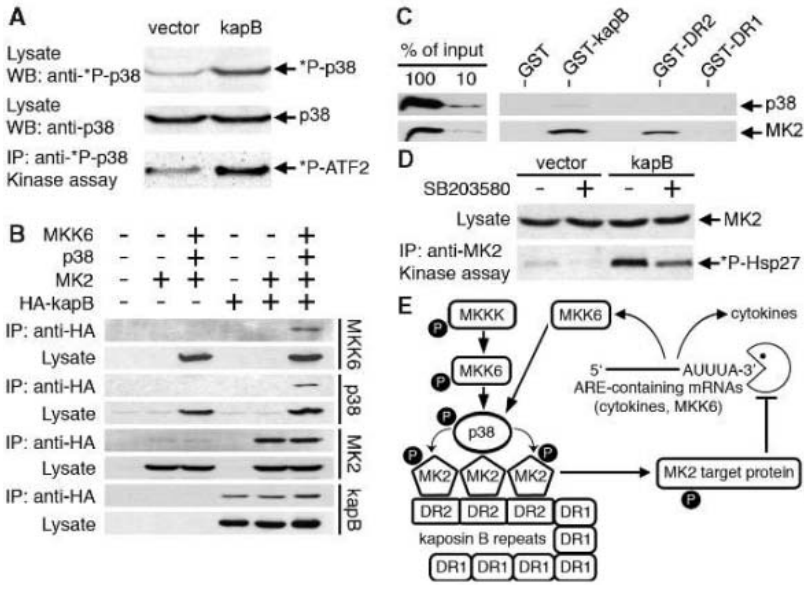

Fig. 4. p38 participates in kaposin B–MK2 signaling complexes. (**A**) HeLa cells were transfected with empty vector or kaposin B expression vector for 30 hours, lysed, and harvested. Portions of the whole-cell lysates were immunoblotted with antibodies to p38 (middle panel) and dual-phosphorylated p38 (upper panel). Dual-phosphorylated p38 was immunoprecipitated from these lysates, and p38 activity was measured with the use of activating transcription factor 2 (ATF2) as a substrate and immunoblotting with antibody to phosphorylated ATF2 (lower panel). (**B**) MKK6 and p38 coimmunoprecipitate with kaposin B–MK2 complexes. 293T cells were transfected with the indicated combinations of hemagglutinin (HA) epitope–tagged kaposin B, MK2, p38, and MKK6 for 48 hours, lysed, and immunoprecipitated with HA mAb. Western blots of the immunoprecipitated material and the whole-cell lysates were probed with polyclonal antibodies to MKK6, p38, MK2, and HA. (**C**) p38 and MK2 proteins (purified from *E. coli*) were incubated overnight at 4°C with the indicated GST-fusion proteins bound to glutathione-Sepharose beads, washed, electrophoresed, and immunoblotted with specific polyclonal antibodies. (**D**) Inhibition of p38 partially blocks kaposin B–mediated increases in MK2 activity. The selective p38 inhibitor SB203580 was added to kaposin B and empty vector transfected HeLa cells for 1 hour, cells were lysed, and whole-cell lysates were immunoblotted with the indicated antibodies. Portions of these lysates were immunoprecipitated with anti-MK2 and assayed for MK2 kinase activity. (**E**) Proposed model of MK2 activation by kaposin B. The reiterated DR2 repeats of kaposin B may bind multiple MK2 proteins in the nucleus, allowing for efficient phosphorylation by p38 MAPK. Phosphorylation of MK2 target proteins results in a blockade in ARE-mediated mRNA degradation, leading to enhanced production of proteins from ARE-containing transcripts. These proteins include cytokines as well as signaling molecules such as MKK6, which in turn could amplify p38 MAPK activation.

activation, leading to further activation of MK2. Much remains to be learned about the mechanisms of MK2 and p38 activation by kaposin B. Binding of MK2 by kaposin B could lead directly to its activation through an induced conformational change; we note that the region on MK2 to which kaposin B binds (Fig. 1D) is consistent with activation via displacement of the inhibitory C-terminal autoregulatory domain of MK2. Bound kaposin B could also shield activated MK2 from the action of cellular phosphatases. As to how p38 becomes activated, because cytokines such as IL-6 can activate the p38 pathway, elevated cytokine release from kaposin B–expressing cells may promote an autocrine or paracrine amplification loop that further enhances p38 activity. Irrespective of its mechanistic details, the finding that kaposin B activates the p38-MK2 pathway forges an important biochemical link between KSHV infection and the enhanced cytokine production that characterizes so many of its associated disease states, and provides a striking example of virus-mediated modulation of mRNA stability.

References and Notes

1. Y. Chang *et al.*, *Science* **266**, 1865 (1994).
2. B. Ensoli, M. Sturzl, *Cytokine Growth Factor Rev.* **9**, 63 (1998).
3. B. Ensoli *et al.*, *Science* **243**, 223 (1989).
4. B. Ensoli, G. Barillari, R. C. Gallo, *Immunol. Rev.* **127**, 147 (1992).
5. S. A. Miles *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4068 (1990).
6. K. V. Komanduri, J. A. Luce, M. S. McGrath, B. G. Herndier, V. L. Ng, *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* **13**, 215 (1996).
7. H. Asou *et al.*, *Blood* **91**, 2475 (1998).
8. H. G. Drexler, C. Meyer, G. Gaidano, A. Carbone, *Leukemia* **13**, 634 (1999).
9. H. G. Drexler, C. C. Uphoff, G. Gaidano, A. Carbone, *Leukemia* **12**, 1507 (1998).
10. N. Nishimoto *et al.*, *Blood* **95**, 56 (2000).
11. K. A. Staskus *et al.*, *J. Virol.* **71**, 715 (1997).
12. R. Sadler *et al.*, *J. Virol.* **73**, 5722 (1999).
13. Single-letter abbreviations for amino acid residues: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.
14. S. Fields, O. Song, *Nature* **340**, 245 (1989).
15. D. Stokoe, K. Engel, D. G. Campbell, P. Cohen, M. Gaestel, *FEBS Lett.* **313**, 307 (1992).
16. W. Meng *et al.*, *J. Biol. Chem.* **277**, 37401 (2002).
17. K. W. Underwood *et al.*, *Structure* **11**, 627 (2003).
18. K. Engel, A. Kotlyarov, M. Gaestel, *EMBO J.* **17**, 3363 (1998).
19. C. Y. Chen, A. B. Shyu, *Trends Biochem. Sci.* **20**, 465 (1995).
20. A. B. Shyu, M. E. Greenberg, J. G. Belasco, *Genes Dev.* **3**, 60 (1989).
21. G. Shaw, R. Kamen, *Cell* **46**, 659 (1986).
22. R. Winzen *et al.*, *EMBO J.* **18**, 4969 (1999).
23. C. Y. Chen, N. Xu, A. B. Shyu, *Mol. Cell. Biol.* **15**, 5777 (1995).

24. Dedicated to the memory of Robert Sadler, Ph.D., who discovered kaposin B as a postdoctoral fellow but died tragically shortly thereafter.

Supporting Online Material

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Figs. S1 to S4

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Mutualistic Fungi Control Crop Diversity in Fungus-Growing Ants

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Leaf-cutting ants rear clonal fungi for food and transmit the fungi from mother to daughter colonies so that symbiont mixing and conflict, which result from competition between genetically different clones, are avoided. Here we show that despite millions of years of predominantly vertical transmission, the domesticated fungi actively reject mycelial fragments from neighboring colonies, and that the strength of these reactions are in proportion to the overall genetic difference between these symbionts. Fungal incompatibility compounds remain intact during ant digestion, so that fecal droplets, which are used for manuring newly grown fungus, elicit similar hostile reactions when applied to symbionts from other colonies. Symbiont control over new mycelial growth by manurial imprinting prevents the rearing of multiple crops in fungus gardens belonging to the same colony.

Ant fungiculture arose in South America about 50 to 60 million years ago in the ancestor of the New World tribe of fungus-growing (attine) ants (*1*). All extant attine ants (~210 described species in 13 genera) are obligately dependent on this symbiosis,

which mostly involves Lepiotaceaeous fungi (order Agaricales, division Basidiomycota) (*1*–*3*). The symbionts of many lower attine ants are closely related to free-living fungi (*4*), but those of *Atta* and *Acromyrmex* leaf-cutting ants have unique coevolved adaptations as a specialized crop (*5*). The ants provide the fungus with fresh substrate and protection against competitors and pathogens (*6*–*8*), and virgin ant queens carry their mother's symbiont when leaving their colony to mate and disperse (*9*). This vertical transmission of the symbiont is expected to stabilize the mutualism by aligning the reproductive interests of the partners (*10*–*12*). However, horizontal exchange of symbionts may occur during cofounding of colonies or symbiont theft after garden loss (*5*, *13*–*16*).

If hostile interactions between symbionts reduce productivity, the introduction of an alien fungus clone will harm not only the resident fungus but also the ant hosts (*10*, *11*, *16*). Ants in full control could possibly avoid these costs by rearing genetically different symbionts in separate nest chambers, but previous studies have suggested that colonies rear only a single fungus clone (*5*, *16*–*18*). This implies that the ants do not have agricultural practices of exchanging crops, nor do they have multiple crops, which are developments that have been essential in human agriculture (*19*). Here we show that single-crop ant farming is actively imposed by the fungal symbiont.

We used fungus gardens (Fig. 1A) that were cultivated by 18 colonies of two sympatric species of Panamanian leaf-cutting ants, *Acromyrmex echinatior* and *A. octospinosus*. These two ant species have never been observed to hybridize, but their clonal fungi belong to the same genetically diverse clade (fig. S1) (*16*, *20*, *21*). Mycelial incompatibility between fungi from different colonies was assessed by inoculating pairs of fungus that were grown 1.5 cm apart on potato dextrose agar (PDA) medium (fig. S2) (*21*). After 2 months, mycelial compatibility could be scored on a scale from 0 to 3 (Fig. 1B) (*21*, *22*). We also obtained genetic fingerprints of the fungi by using amplified fragment length polymorphism (AFLP) to estimate relative genetic distance (percentage of bands not homologous) between pairs of clones and to confirm that each source colony cultivated a single clone (*21*). The latter was done by AFLP analysis of five to six independently isolated samples of the same fungus garden from each of 10 colonies. Alignment of these profiles showed identical banding patterns within fungus gardens (*21*).

Mycelial incompatibility was highly variable among pairs of clones (Fig. 1B) and was positively correlated with genetic distance (Mantel test: $r = 0.855$, $P < 0.0001$) (Fig. 1C). Average reactions within ant species (1.40 for *A. echinatior* and 1.54 for *A. octospinosus*) were less hostile than between species (1.98). This result corresponded to lower relative genetic distances between fungi within (0.20 for *A. echinatior* and 0.23 for *A. octospinosus*) than between ant species (0.29). An effect likelihood ratio

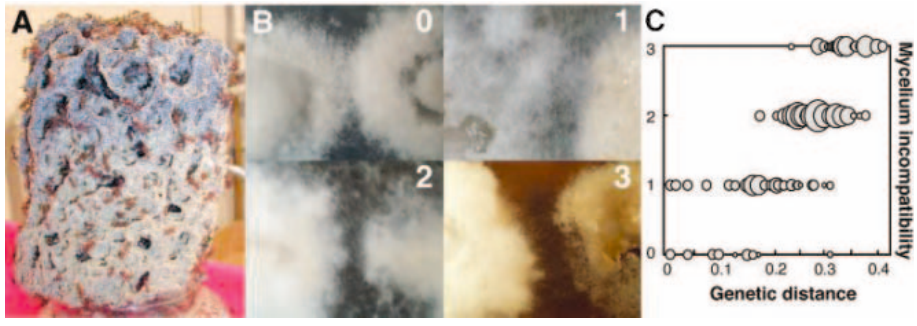

Fig. 1. (**A**) An *A. octospinosus* fungus garden and (**B**) typical examples of the four degrees of mycelial incompatibility between fungus clones in vitro. 0 shows fully compatible clones with no demarcation zone; 1 shows weakly incompatible with a slight, but clearly visible, demarcation zone; 2 shows incompatible clones with a distinct demarcation zone; and 3 shows strongly incompatible clones with a broad demarcation zone and brown coloration of mycelium and/or medium. (**C**) The relation between pairwise degree of incompatibility and genetic distance (171 different combinations, each replicated six times) (fig. S2). The area of each circle is proportional to the number of replicate combinations producing a given result. Controls are not shown, becauses they were all fully compatible (*21*).

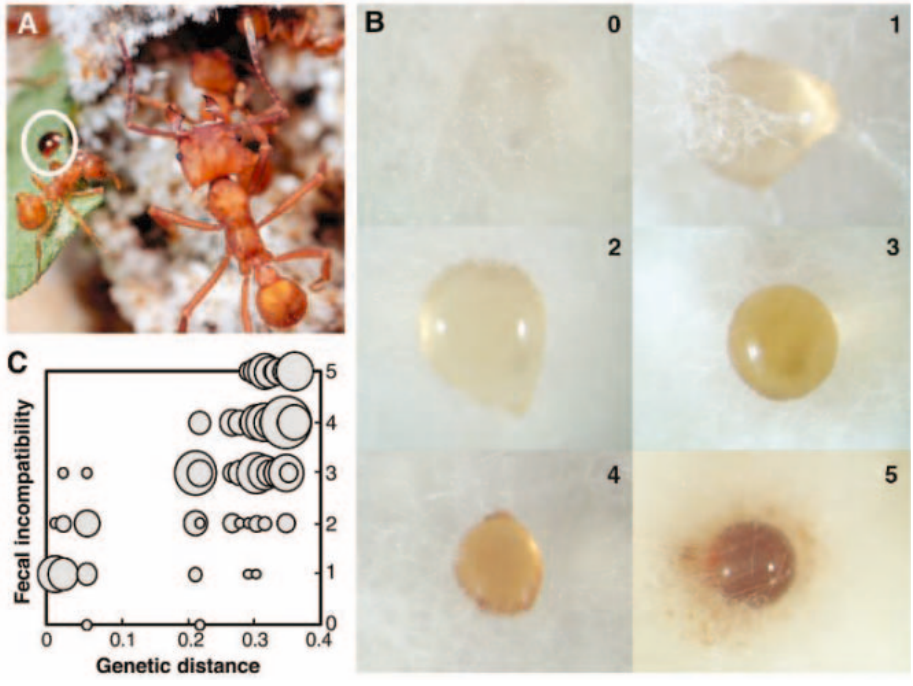

Fig. 2. (**A**) An *A. echinatior* fungus garden with a fecal droplet (encircled) [photo courtesy of Klaus Lechner]. (**B**) Typical examples of the categories of reaction of plated fungi (after 24 hours) (*21*) toward fecal droplets produced by workers from other colonies of *A. echinatior* or *A. octospinosus* from the same site. 0 shows complete absorption indicating full compatibility; 1 shows partial absorption; 2 shows distinct mycelium growth on the droplet but no absorption; 3 shows no mycelial growth on the droplet or in the direct vicinity of the droplet; 4 shows active avoidance of the droplet by fungal hyphae, with droplet turning light to dark brown; and 5 shows complete rejection of the droplet and very dark coloration of both droplet and surrounding mycelium. (**C**) The pairwise degree of incompatibility between fungal fragments maintained by ants and fecal droplets after 24 hours, plotted against genetic distance (garden fragments and fecal droplets from eight different colonies were combined in all possible ways and replicated eight times). Circle area is proportional to number of combinations producing a given result and controls are not shown [67.9% showed full compatibility (0); 30.4% showed partial absorption (1); and 1.7% showed distinct mycelium growth on the droplet, but no absorption (2)] (*21*).

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(ELR) test confirmed that incompatibility is related to fungal genetic distance ($\chi^2 = 2569$, degrees of freedom (df) = 1, $P < 0.0001$) and not an effect of ant species combination ($\chi^2 = 2.199$, df = 2, $P = 0.3330$) (*21*).

Acromyrmex worker ants actively discriminate between fragments of their resident fungus and genetically different symbionts (*16*), which confirms predictions that the ants have a strong short-term interest in maintaining genetically pure gardens (*11*). The ants reject alien fungus fragments for at least 1 week after being deprived of their resident garden, which indicates that avoidance of symbiont competition, at least initially, overrides symbiont replacement behavior, which is in their long-term interest (*16*). This suggests that the weeding behavior of worker ants may be directly linked to fungal incompatibility compounds.

All fungus-growing ants manure newly grown mycelium with their own feces (*23*, *24*). In this process, fungal enzymes, which help break down plant material, pass through the ant gut into fecal droplets (*25*), which are deposited on fresh leaves (Fig. 2A) or directly on the fungus garden, where they are readily absorbed (*2*). If incompatibility compounds remained unaffected by ant digestion, we would expect that fecal droplets secondarily might have become defensive extended phenotypes of the resident symbiont. We tested this idea by examining the reactions of fungus and ants toward fecal droplets from other colonies when the droplets were applied to fungus garden fragments of live experimental colonies (fig. S3) (*21*).

Incompatibility reactions of fungi toward fecal droplets from nonresident ants were scored on a scale ranging from 0 to 5 after 24 hours (Fig. 2B). There was a significant association between the intensity of the reactions and the genetic distance between the resident fungus and the fecal droplet–producing fungus (Mantel test: $r = 0.960$, $P < 0.0001$) (Fig. 2C). ELR testing showed that the magnitude of this effect was not due to ant species combination ($\chi^2 = 1.357$, df = 2, $P = 0.5074$) but only to the genetic distance of symbionts ($\chi^2 = 432.4$, df = 1, $P < 0.0001$) (*21*).

In the same experimental colonies, there was a significant association between the likelihood that ants would remove alien fecal droplets and the genetic distance between the resident and the droplet-producing fungus (Mantel test: $r = 0.721$, $P < 0.0001$) (Fig. 3A) and the droplet-fungus incompatibility reaction (Mantel test: $r = 0.651$, $P < 0.0001$) (Fig. 3B). Weeding behavior around the droplets, which resulted in the accumulation of additional fungal waste material (*8*, *21*), was associated with the same predictor variables [Mantel tests: $r = 0.700$, $P < 0.0001$ (Fig. 3C) and $r = 0.686$, $P < 0.0001$ (Fig. 3D)]. Droplet removal and garbage accumulation were likewise correlated (Mantel test: $r = 0.728$, $P < 0.0001$) (*21*). This suggests that the ants actively discard alien fecal droplets and any mycelium that has been in touch with these droplets.

In a final experiment, we found that initially incompatible interactions between fecal droplets and fungi became compatible when ants were forced to feed on an incompatible alien symbiont for 10 days. The ants' new fecal droplets also became incompatible with their original resident fungus (Fig. 4, B and C) (*21*). An ELR test showed a significant main effect of the ant fungus combination ($\chi^2 = 9.498$, df = 1, $P = 0.0021$) and a significant interaction between the ant fungus combination and time ($\chi^2 = 11.58$, df = 1, $P = 0.0007$) (*21*). Controls did not show significant changes in compatibility, which confirmed that the compounds responsible for incompatibility reactions are exclusively fungus-derived.

The parallel reactions of resident fungi toward alien mycelia (Fig. 1) and fungus-derived fecal droplets (Figs. 2 and 4), and the hostile ant behavior toward alien droplets (Fig. 3) and alien fungus fragments (*16*), show that the host ants and their resident symbiont jointly prevent the introduction of competing fungus clones, which agrees with evolutionary theory on host symbiont conflict over symbiont mixing (*10*, *11*). Incompatibility reactions (brown coloration of mycelium) have also been observed in interactions between the ants' fungal symbionts and pathogenic fungi (*26*, *27*) and probably facilitate the detection of fungal disease. However, the present results suggest that these recognition abilities have been enhanced after fungus domestication, particularly in the higher attine ants (*3*, *5*). Direct assessments by the ants (*16*, *28*, *29*) and the derived recognition mechanism by the fecal droplets (<24 hours; Figs. 2 and 4) are much faster than recognition through mycelial contact, which takes 5.6 days on average (range, 3 to 7 days), even if tufts of alien mycelium are applied directly on mats of plated symbionts (Fig. 1B) (*21*). The chemical nature of the incompatibility compounds remains unknown, but they could be similar to those of free-living basidiomycetes or be derivates of evolutionary-derived enzymes of the symbionts (*30*).

Neither direct recognition of alien symbionts by the ants nor mycelial incompatibility can explain why *Acromyrmex* leaf-cutting ants are restricted to rear a single symbiont clone per colony. Abundant genetic variation between clones is available, and mature nests of *A. octospinosus* and *A. echinatior* are usually compartmentalized with multiple gardens that could keep symbiont strains separated. The present study suggests that

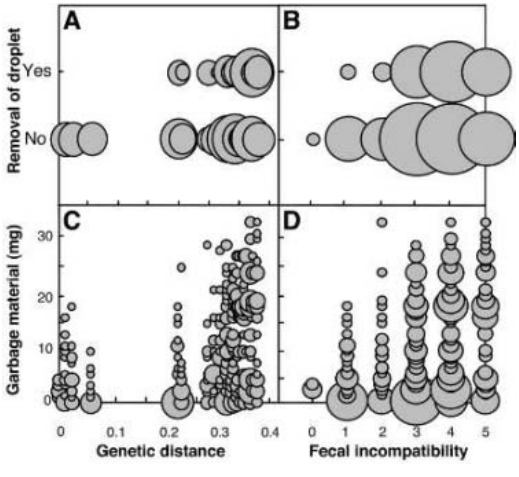

Fig. 3. (**A** and **B**) Fecal droplet removal and (**C** and **D**) amount of garbage (milligrams of fresh weight) deposited 24 hours after application of fecal droplets that were obtained from the same eight-by-eight combinations of fungus gardens and ants used in Fig. 2 (*21*). Data are plotted against genetic distance between the resident and the droplet-producing fungus [(A) and (C)] and the degree of fecal-droplet incompatibility (0 to 5) [(B) and (D)]. Circle area is proportional to the number of combinations producing a given result. Controls are not plotted, because resident fecal droplets were never removed and the average amount of garbage deposited was 1 ± 0.3 mg (mean ± SE), which was substantially less than the mean garbage accumulation of all the test combinations (4 ± 0.4 mg) (*21*).

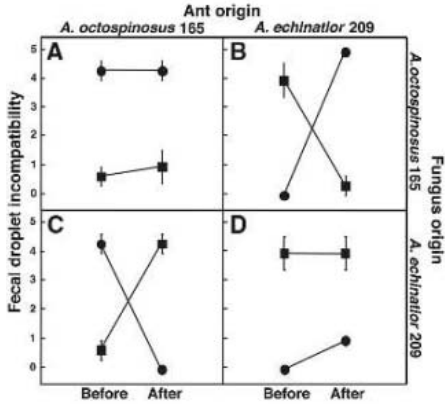

Fig. 4. Fungus incompatibility reactions toward resident and foreign fecal droplets, before and after force-feeding the ants that produced the droplets for 10 days. Two colonies (Ao165 and Ae209), which were initially droplet-incompatible (Fig. 3), were used for reciprocal force-feeding treatments (**B** and **C**) and controls (**A** and **D**). Mean reactions ± SE (n = 3 replicate pairings) displayed by fungus Ao165 (squares) and Ae209 (circles) are plotted.

the ants' manuring practice is the decisive factor that constrains colonies to rearing a single clone of symbiont. Obligate manuring with ant feces allows the resident fungus to control the genetic identity of new gardens in the nest, causing the removal of unrelated fungi before they contribute to ant feeding and the production of compatible fecal droplets (Fig. 4). The manurial imprint of the fungus therefore makes ant agriculture dependent on a single symbiont that is very difficult to replace and impossible to combine with secondarily acquired symbionts.

The manurial imprint of fecal droplets is a clear example of fungal signaling affecting ant behavior and is possibly more important than suggested fungal signaling for directing the choice of food plants by workers to avoid an overdose of toxins (*31*, *32*). Similar manipulative effects of resident fungi toward competing clones may have convergently evolved in the fungus-growing termites, where the fungus substrate is entirely derived from fecal matter (*33*). This could explain why fungus-growing termites also rear single clones of fungus, despite initiating colonies with horizontally acquired fungal spores (*33*, *34*). None of these constraints have ever applied to human agriculture because crops and expertise have been culturally transmitted between tribes from the beginning of human farming (*35*).

References and Notes

1. I. H. Chapela, S. A. Rehner, T. R. Schultz, U. G. Mueller, *Science* **266**, 1691 (1994).
2. N. A. Weber, Ed., *Gardening Ants: The Attines* (American Philosophical Society, Philadelphia, 1972).
3. T. R. Schultz, U. G. Mueller, C. R. Currie, S. A. Rehner, in *Ecological and Evolutionary Advances in Insect-Fungal Associations*, F. Vega, M. Blackwell, Eds. (Oxford Univ. Press, Oxford, 2004), pp. 149–190.
4. P. Villesen, U. G. Mueller, T. R. Schultz, R. M. M. Adams, A. C. Bouck, *Evolution* **58**, 2252 (2004).
5. U. G. Mueller, S. A. Rehner, T. R. Schultz, *Science* **281**, 2034 (1998).
6. M. Bass, J. M. Cherrett, *Ecol. Entomol.* **19**, 215 (1994).
7. R. D. North, C. W. Jackson, P. E. Howse, *Trends Ecol. Evol.* **12**, 386 (1997).
8. C. R. Currie, A. Stuart, *Proc. R. Soc. London Ser. B* **268**, 1033 (2001).
9. B. Hölldobler, E. O. Wilson, *The Ants* (Springer Verlag, Berlin, 1990).
10. S. A. Frank, *Proc. R. Soc. London Ser. B* **263**, 339 (1996).
11. S. A. Frank, *Evolution* **57**, 693 (2003).
12. E. A. Herre, N. Knowlton, U. G. Mueller, S. A. Rehner, *Trends Ecol. Evol.* **14**, 49 (1999).
13. S. W. Rissing, G. B. Pollock, M. R. Higgins, R. H. Hagen, D. R. Smith, *Nature* **338**, 420 (1989).
14. D. Bekkevold, J. Frydenberg, J. J. Boomsma, *Behav. Ecol. Sociobiol.* **46**, 103 (1999).
15. R. M. M. Adams, U. G. Mueller, A. K. Holloway, A. M. Green, J. Narozniak, *Naturwissenschaften* **87**, 491 (2000).
16. A. N. M. Bot, S. A. Rehner, J. J. Boomsma, *Evolution* **55**, 1980 (2001).
17. A. M. Green, U. G. Mueller, R. M. M. Adams, *Mol. Ecol.* **11**, 191 (2002).
18. U. G. Mueller, S. E. Lipari, M. G. Milgroom, *Mol. Ecol.* **5**, 119 (1996).
19. Y. Zhu *et al.*, *Nature* **406**, 718 (2000).
20. T. D. Schultz, D. Bekkevold, J. J. Boomsma, *Insect Soc.* **45**, 457 (1998).
21. Materials and methods are available as supporting material on *Science* Online.
22. E. M. Hansen, J. Stenlid, M. Johansson, *Mycol. Res.* **97**, 1229 (1993).
23. M. M. Martin, *Science* **169**, 15 (1970).
24. T. Murakami, S. Higashi, *J. Ethol.* **15**, 17 (1997).
25. S. Rønhede, J. J. Boomsma, S. Rosendahl, *Mycol. Res.* **108**, 101 (2004).
26. C. R. Currie, personal communication.
27. M. Poulsen, personal observation.
28. A. M. M. Viana *et al.*, *Chemoecology* **11**, 29 (2001).
29. U. G. Mueller, J. Poulin, R. M. M. Adams, *Behav. Ecol.* **15**, 357 (2004).
30. J. J. Worrall, *Mycologia* **89**, 24 (1997).
31. P. Ridley, P. E. Howse, C. W. Jackson, *Experentia* (*Basel*) **52**, 631 (1996).
32. U. G. Mueller, *Am. Nat.* **160** (suppl., 67 (2002).
33. D. K. Aanen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 14887 (2002).
34. H. Katoh, T. Miura, K. Maekawa, N. Shinzato, T. Matsumoto, *Mol. Ecol.* **11**, 1565 (2002).
35. J. Diamond, *Science* **281**, 1974 (1998).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/307/5710/741/DC1
Materials and Methods
Figs. S1 to S3
References

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JACK UETRECHT, CHAIR
THOMAS KAWABATA, VICE CHAIR

- **Keynote: The Danger Hypothesis and Adverse Drug Reactions**
  (Polly Matzinger)
- **Mechanistic Aspects of Idiosyncratic Drug Reactions**
  (Kevin Park / Werner Pichler / Lance Pohl / Craig Svensson)
- **Basic Immunology with Relevance to Adverse Drug Reactions**
  (Cynthia Ju / Juan LaFaille / Andrew Fontenot)
- **Predictive Screens**
  (Tim Ryan / Bruce Car / Greg Slatter / Randy Peterson)
- **Role of Regulatory Agencies in Encouraging and Enabling Research in Idiosyncratic Drug Reactions**
  (Kenneth Hastings / John Senior)
- **Hepatotoxicity**
  (Paul Watkins / Chris Day / Dan Burns / James Boyer)
- **Selected Abstracts for Oral Presentation**
  (*Thomas Kawabata*)
- **Pharmacogenetics**
  (Michel Eichelbaum / Munir Pirmohamed / Matthias Schwab / Leif Bertilsson)
- **Drug-Induced Long QT Syndrome**
  (Peter Siegl / John Mitcheson / Borje Darpo)

ANALYTICAL CHEMISTRY
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- **Microanalytical Systems I: Fluidics**
  (*Dick Crooks* / Andreas Manz / Thomas Laurell)
- **Microanalytical Systems II: Cells**
  (*Jörg P. Kutter* / Jon M. Cooper / Elisabeth Verpoorte / Helene Andersson)
- **Microanalytical Systems III: Nanofluidics**
  (*Elizabeth Zubritsky* / J. Michael Ramsey / Paul Bohn)
- **Microanalytical Systems IV: Systems**
  (*Andrew deMello* / Antonio J. Ricco / Tom van de Goor / Takehiko Kitamori)
- **BioMEMs & MEMS**
  (*Yoshinobu Baba* / Andreas Hierlemann / Alexandra Fuchs)
- **Nanowires**(*Claire Darby* / Donald Fitzmaurice / Reginald Penner / Bernadette Quinn)
- **Nanopores**
  (*Li Sun* / Lydia Sohn / Henry S. White)
- **Microarrays**
  (*Larry Morrison* / John T. McDevitt / Hans Lehrach / Robert M. Corn)
- **Open Session**
  (*D. Jed Harrison*)

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- **Blood Vessel Guidance**
  (*Federico Bussolino* / Christer Betzholtz / David Anderson / Anne Eichman)
- **Bone Marrow Derived Hemangiogenic Progenitors**
  (Shahin Raffii / Eli Keshet / Stephanie Dimmeler)
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  (*Kari Alitalo* / Brant Weinstein / Peter Carmeliet / Didier Stainier)
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- **Growth Factors and Their Signalling Pathways**
  (*Harold F. Dvorak* / Kari Alitalo / Napoleone Ferrara / Masabumi Shibuya / David Cheresh / Donald McDonald)
- **The Influence of Microenvironment in Vascular Morphogenesis**
  (*Michael Gimbrone* / Richard Hynes / Luisa Iruela Arispe / Hellmut Augustin / Raghu Kalluri)
- **Paracrine Cellular Signalling**
  (*Denisa Wagner* / Pat D'Amore / William Sessa / Jeff Pollard)
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  (*Judah Folkman* / Rakesh Jain / Gabriele Bergers / Doug Hanahan)
- **Therapeutic Applications**
  (*David Peritt* / Patrizia Rovere-Querini)

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  (*Patrick Williamson* / Shigekazu Nagata / Glen Matsushima)
- **Recognition in Invertebrates**
  (*Yoshinobu Nakanishi* / Michael Hengartner / Akiko Shiratsuchi / Loriano Ballarin)
- **Diversity of Recognition Mechanisms**
  (*Valerian Kagan* / Eric Baehrecke)
- **Phagocyte Engulfment Mechanisms**
  (*Michael Hengartner* / Kodi Ravichandran / Simon Brown)
- **Alternative Phagocytes**
  (*Kodi Ravachandran* / Raymond Birge / Jeffrey Curtis)
- **Apoptotic Cell Recognition and Disease**
  (*Giovanna Chimini* / Silvia Finnemann / Marcello Barcinski)

APPLIED & ENVIRONMENTAL MICROBIOLOGY
CONNECTICUT COLLEGE
NEW LONDON, CT
JUL 24-29, 2005
GERARD MUYZER, CHAIR
KENNETH NEALSON, VICE CHAIR

- **The Need for Microbial Ecophysiology**
  (*Doug Capone* / Gijs Kuenen)
- **Cultivating the Uncultured**
  (*Harold Drake* / Slava Epstein / Karsten Zengler / Martin Hahn / Jared Leadbetter)
- **Novel Microbial Metabolisms**
  (*Svetlana Dedysh* / Oded Béjà / Marc Strous)
- **Sustainable Applications of Industrial Biotechnology**
  (*Claudia Schmidt-Dannert* / Bernard Hauer / Douglas Cameron / Lonnie Ingram / Uwe Sauer)
- **Patterns in Microbial Diversity**
  (*Matthew Kane* / Lise Øvreas / Brendan Bohannan)
- **Microbial Consortia and Symbioses**
  (*Nicole Dubilier* / Edward Ruby / Jörg Overmann / Shana Goffredi / Andreas Brune)
- **Ecology of Aquatic Viruses**
  (*John Paul* / Curtis Suttle / Markus Weinbauer / Corina Brussaard)
- **Metagenomics in Applied and Environmental Microbiology**
  (*Jürgen Eck* / Tom Isenbarger / Gene Tyson / Rolf Daniel / Rachel Poretsky)
- **Special Lecture**
  (*Ken Nealson* / Craig Venter)

ARCHAEA: ECOLOGY, METABOLISM & MOLECULAR BIOLOGY
MAGDALEN COLLEGE
OXFORD, UK
AUG 14-19, 2005
PAUL BLUM &
JOHN VAN DER OOST, CO-CHAIRS
IMKE SCHROEDER &
MALCOLM WHITE, CO-VICE CHAIRS

- **Key Note Talk & Archaeal Virology**
  (Karl Stetter / Mark Young / David Prangishvili)
- **Evolutionary Genomics**
  (*Mark Young* / Eugene Koonin / James Lake / Patrick Forterre / Xu Peng)
- **Functional Genomics & Systems Biology**
  (Mike Adams / Rolf Bernander / Greg Ferry)
- **Biomedicine & Biotechnology**
  (John Williams / Shiladitya DasSarma / Dennis Sprott / Jonathan Trent / Robert Kelly / Paul Eckburg)
- **RNA Synthesis & Degradation**
  (*Dieter Soll* / John Reeve / Michael Thomm / Elena Evguenieva-Hackenberg / Charles Daniels / Mohammed Ouhammouch / Felicitas Pfeiffer)
- **DNA Replication, Repair & Recombination**
  (Malcolm White / Stephen Bell / Takehiko Nohmi / Francesca Pisani / Thorston Allers)
- **Protein Synthesis, Degradation & RNA Modification**
  (*Patrick Dennis* / Dieter Soll / Julie Maupin-Furlow / Paola Londei / Arina Omer)
- **Metabolism**
  (*Richard Shand* / Betina Siebers / Imke Schroeder / Mechke Pohlschroder / John Leigh)
- **Archaeal Ecology & Diversity**
  (*Karl Stetter* / Edward Delong / Richard Seifert / Robert Goodman / Christa Schleper / Reinhard Rachel)

ASSISTED CIRCULATION
BIG SKY RESORT
BIG SKY, MT
AUG 21-26, 2005
ERIC ROSE, CHAIR
ALAN SNYDER, VICE CHAIR

- **Intro Session**
  (*Eric Rose* / *Marvin Konstam* / Leslie Miller / Sharon Hunt / James Long)
- **Present and Emerging Technologies**
  (*Alan Snyder* / *James Anderson* / Robert Kormos / William Wagner / Gerson Rosenberg)
- **Preventing and Managing Adverse Events (1)**
  (*William Holman* / *Mariel Jessup* / Frank Lowy)
- **Preventing and Managing Adverse Events (2)**
  (*Lynn Warner Stevenson* / *Ann Marie Schmidt* / Robert Robbins / Sam Goldhaber / Diane Meier)
- **Clinical Trials**
  (*Sean Tunis* / *Annetine Gelijns* / Alan Moskowitz / Daniel Heitjan)
- **Challenges to Technological Innovations**
  (*John Watson* / *Beverly Lorell* / John Woodard / Paul Citron / Timothy Baldwin)
- **Recovery**
  (*Robert Kormos* / Guillermo Torre / Christine Morevick)
- **Cell Transplantation**
  (*Silviu Itescu* / *Richard Weisel* / Kenneth Chien / Katherine Verfaille / Martin Leon)

ATHEROSCLEROSIS
KIMBALL UNION ACADEMY
MERIDEN, NH
JUN 19-24, 2005
ISRAEL CHARO &
ALAN DAUGHERTY, CO-CHAIRS
ELIZABETH NABEL, VICE CHAIR

- **Immune Function**
  (*Goran K. Hansson* / Goran K. Hansson / Ziad Mallat / Joseph L. Witztum / Amy Major / Stewart Whittman)
- **Inflammatory Processes**
  (*Peter Libby* / Moshe Arditi / Jeanine D'Armiento / Peter Libby / Mark B. Pepys / Rama Natarajan / Dan Simon)
- **Thrombotic and Hemostatic Mechanisms**
  (*Shaun R. Coughlin* / Shaun R. Coughlin / Mark B. Taubman / Bruce Furie)

- **Lipoprotein Metabolism**
  (*Mary G. Sorci-Thomas* / Miranda van Eck / Daniel J. Rader / Gwendalyn Randolph / Alan M. Fogelman / Michael J. Thomas / Vasanthy Narayanaswami)
- **Metabolic Syndrome and Atherosclerosis**
  (*Doug Vaughn* / Peter Tontonoz / Tony Ferrante)
- **Macrophage Biology**
  (*Martha K. Cathcart* / Catherine C. Hedrick / Elaine Raines / William Muller / Ara Aslanian)
- **Vascular Wall Stem Cell Biology**
  (*Elizabeth G. Nabel* / Victor Dzau / Shanin Rafii)
- **Cell Biology of Atherosclerosis**
  (*Joseph Loscalzo* / Bradford C. Berk / Cecelia M. Giachelli / Thomas Quertermous / Dennis Bruemmer)
- **Human and Animal Genetics of Atherosclerosis**
  (*Aldons J. Lusis* / Paivi Pajukanta / Jonathan Smith / Alan Attie)

ATMOSPHERIC CHEMISTRY
BIG SKY RESORT
BIG SKY, MT
SEP 4-9, 2005
DAVID FAHEY, CHAIR
DOUGLAS WORSNOP, VICE CHAIR

- **Key Perspectives**
  (*Kenneth Demerjian* / Susan Solomon / Spyros Pandis)
- **Measuring and Modeling Aerosols and Their Impact on Climate**
  (*Kimberly Prather* / Jose-Luis Jimenez / Barbara Turpin / Yinon Rudich / Joyce Penner)
- **Aerosols and Clouds**
  (*Graham Feingold* / John Seinfeld / Sonia Kreidenweis / Thomas Peter)
- **Urban Pollution, Ozone Formation, and Regional Air Quality**
  (*Mario Molina* / *Fred Fehsenfeld* / Min Shao / Yutaka Kondo / Paul Crutzen / Robert Harley / Joost de Gouw)
- **Laboratory Studies of Atmospheric Processes**
  (*Neil Donahue* / Edward Lovejoy / Michael Pilling)
- **The Biosphere**
  (*Mary Anne Carroll* / Elizabeth Holland)
- **Measurements from Space**
  (*Anne Douglass* / Daniel Jacob / Randall Martin)
- **Global Modeling**
  (*Jose Rodriguez* / Mark Lawrence / Michael Trainer / Michael Prather)

ATOMIC PHYSICS
TILTON SCHOOL
TILTON, NH
JUN 26-JUL 1, 2005
ERIC CORNELL, CHAIR
CHRIS MONROE, VICE CHAIR

- **Quantum Information**
  (*Michael Chapman* / Luming Duan / Alex Kuzmich / Dietrich Leibfried)
- **Bose-Einstein Condensation**
  (*Keith Burnett* / Lene Hau / Massimo Inguscio / David Weiss)
- **Degenerate Fermi Gases**
  (*Wolfgang Ketterle* / Christoph Salomon / Cindy Regal)
- **Condensed Matter Connections**
  (*Anders Sorensen* / Rob Shoelkopf)
- **Precision Measurements**
  (*Larry Hunter* / Dmitri Budker / Eric Hessels)
- **Ultrafast and High Field Physics**
  (*Todd Ditmire* / Margaret Murnane)
- **Quantum Control**
  (*David Tannor* / Robert Jones / Dieter Meschede / Paul Haljan)
- **Applications**
  (*David Pritchard* / Naomi Halas)

BARRIER FUNCTION OF MAMMALIAN SKIN
MOUNT HOLYOKE COLLEGE
SOUTH HADLEY, MA
AUG 7-12, 2005
STEVEN HOATH &
GERALD KASTING, CO-CHAIRS
ANTHONY RAWLINGS &
MIKE ROBERTS, CO-VICE CHAIRS

- **Comparative Biology and Physics of the Epidermal Barrier**
  (*Walt Holleran* / Gopi Menon / Bob Lochhead)
- **Membrane Structures and Transporters**
  (*Peter Elias* / Gerd Schmitz / Andreas Stahl / Lily Bourgignon / Johanna Brand)
- **Nanoscale Characterization of Skin Permeability**
  (*Mike Roberts* / Johannes Nitsche / Daniel Blankschtein / Yuri Anissimov)
- **Water and the Stratum Corneum**
  (*Joachim Fluhr* / Tony Rawlings / Peter Elias)
- **Novel Analytical Techniques**
  (*Richard Guy* / Samir Mitragotri / Stephen Hendrix)
- **Imaging the Stratum Corneum**
  (*Phil Wertz* / Lars Norlen / Marek Haftek / Stig Ollmar / Roger Wepf)
- **Hot Topics**
  (*Manige Fartasch*)
- **Follicular Penetration**
  (*Juergen Lademann* / Nina Otberg / Joke Bouwstra / Michael Bonner)
- **Debate: Does TEWL Correlate to Epidermal Barrier Properties?**
  (*Randy Wickett* / Joachim Fluhr / Bob Chilcott)

BIOINFORMATICS:
FROM PREDICTIVE MODELS TO INFERENCE
COLBY COLLEGE
WATERVILLE, ME
AUG 7-12, 2005
DREW ENDY, CHAIR
EDWARD MARCOTTE, VICE CHAIR

- **What is Biological Information?**
- **Timescales of Biological Information Processing**
- **Environmental Information**
- **Genetic Information**
- **Epigenetic Information**

BIOLOGICAL MOLECULES IN THE GAS PHASE
BATES COLLEGE
LEWISTON, ME
JUL 24-29, 2005
MATTANJAH DEVRIES, CHAIR
ALBERT HECK, VICE CHAIR

- **Protein Misfolding and Amyloid Formation**
  (*Mike Bowers* / David Teplov / Alison Ashcroft / Andrea Sinz)
- **Folding and Conformation**
  (*David Pratt* / Joan-Emma Shea / Sven Hovmoller / Martin Jarrold)
- **Single Molecules**
  (Gilad Haran / X. Sunney Xie / Martin Zanni / Sander Woutersen)
- **Spectroscopy I**
  (*Tim Zwier* / Lavina Snoek / Michel Mons / Isabelle Compagnon)
- **Spectroscopy II**
  (*Karl Kleinnermans* / Anne Zehnacker-Rentien / Wei Kong / Andrzej L. Sobolewski)
- **Spectroscopy of DNA**
  (*Jerry Spooner* / Brian Kohler / Glake Hill / Seong Keun Kim)
- **Top Down Approaches to Protein Structure I**
  (*Evan Williams* / Christoffer Borchers / Scott McLuckey / Kathryn Breuker)
- **Top Down Approaches to Protein Structure II**
  (*Albert Heck* / Neil Kelleher / Ron Heeren / Don Hunt)
- **Feature Lecture**
  (Fred McLafferty)

BIOMATERIALS:
BIOCOMPATIBILITY / TISSUE ENGINEERING
HOLDERNESS SCHOOL
PLYMOUTH, NH
JUL 31-AUG 5, 2005
KEVIN HEALY, CHAIR
ANDRES GARCIA, VICE CHAIR

- **Lessons Learned: Where Do We Go from Here?**
  (*Kevin E. Healy* / David F. Williams / Buddy D. Ratner)
- **Cell-Material Interactions**
  (*Andrés Garcia* / Christopher Chen / Dennis Discher / Joyce Wong)
- **Host Response-Patient Variability**
  (*Jonathan Black* / Steve Goldring / Joshua J. Jacobs)
- **In Vivo Function of Tissue Equivalents**
  (*James M. Anderson* / Tony Atala / Laura E. Niklason / Stephen F. Badylak)
- **Protein Adsorption**
  (*Igal Szleifer* / Erwin A. Vogler / Tom Horbett)
- **Materials-Dependent In Situ Tissue Regeneration**
  (*William Wagner* / Jeffery A. Hubbell / Kristi Anseth / David Mooney)
- **Wound Healing in Context**
  (Elazer R. Edelman / Julia Babensee)
- **Advances in Biomaterials Science**
  (*D.W. Grainger* / David Tirrell / Mark W. Grinstaff / Heather Maynard)
- **Stem Cell Therapy - Hype or Reality**

BIOORGANIC CHEMISTRY
PROCTOR ACADEMY
ANDOVER, NH
JUN 12-17, 2005
KURT DESHAYES & NEIL MARSH, CO-CHAIRS
LESLIE SLOAN &
PETER TONGE, CO-VICE CHAIRS

- **Tinkering with the Central Paradigm**
  (Scott Silverman / Christine Chow / Jack Szostak)
- **Nature Mimicked**
  (Virgil Persec / Annelise Barron / Alan Kennan / Stefan Matile)
- **Nature Redesigned**
  (Trevor Douglas / Lynne Regan / Barrie Wilkinson)
- **Signals and Pathways**
  (Alice Ting / Maurizio Pellecchia / Thomas Engler / Linda Hsieh-Wilson)
- **Enzymes and Biosynthesis**
  (Mark Distefano / Judith Klinman / Craig Townsend)
- **Chemical Tools**
  (Tim Dore / David Berkowitz / Bradley Smith / M.G. Finn)
- **Diseases and Therapies I**
  (Peter Toogood / Ranabir Sinha Roy / Christian Wiesman / Clifton Barry)
- **Diseases and Therapies II**
  (Celia Schiffer / Greg Weiss / Adrian Whittey)
- **New Paradigms, New Technologies**
  (Henrik Pederson / Chad Mirkin / Stuart Schreiber)

BONES & TEETH
KIMBALL UNION ACADEMY
MERIDEN, NH
JUL 10-15, 2005
RENE ST-ARNAUD, CHAIR
PAMELA ROBEY, VICE CHAIR

- **New Developments**
  (*Gérard Karsenty* / Ronen Schweitzer / Paolo Bianco)
- **Transcriptional Control of Bone Cell Differentiation and Function**
  (*Patricia Ducy* / Ernestina Schipani / Hiroshi Takayanagi / Donald Glass)
- **Advances in Stem Cell Biology: From the Inner Cell Mass to the Post-Natal Organism**
  (*Pamela G. Robey*)
- **Mineral Homeostasis and Calcification**
  (*L. Darryl Quarles* / Robert Terkeltaub / Lynda Bonewald / Paul Price / Dwight Towler)
- **Teeth / Craniofacial Biology**
  (*Marc McKee* / Irma Thesleff / Michael Paine / Songtao Shi)
- **Signaling / Cytokines**
  (*Matthew Gillespie* / Robyn Starr / Toshiyuki Takai / Roland Baron)
- **Osteoclast / Bone Resorption**
  (*Robert L. Jilka* / Brendan Boyce / Stavroula Kousteni / Mitch Schaffler)
- **Skeletal Anabolic Agents**
  (*Nicola C. Partridge* / Sundeep Koshia / Lin Qin / Ross Garrett / Peter Bodine)
- **Keynote Lecture**
  (*René St-Arnaud* / Henry Lee)

CAG TRIPLET REPEAT DISORDERS
MOUNT HOLYOKE COLLEGE
SOUTH HADLEY, MA
JUL 24-29, 2005
MICHAEL LEVINE, CHAIR
DIANE MERRY, VICE CHAIR

- **Clinical Presentation and Neuropathology**
  (Sarah J. Tabrizi / Richard L.M. Faull / *Gillian Bates* / *Leslie Thompson*)
- **Polyglutamine Impact on the Cytoskeleton and Axonal Transport**
  (Larry Goldstein / Cynthia McMurray / *Kurt Fishbeck* / *Nancy Bonini*)
- **Data Blitz and New Developments**
  (TBA - from posters / *Jang-Ho Cha*)
- **Normal Function of Polyglutamine Proteins and Impact on Pathogenesis**
  (Randall N. Pittman / Sylvia Krobitsch / *Harry Orr* / *Al La Spada*)
- **Neuronal Interactions in Polyglutamine Diseases and Excitotoxicity**
  (Lynn Raymond / Paolo Calabresi / William Yang / *Janet Dubinsky* / *Kerry Murphy*)
- **Triplet Repeats in Non-Coding Regions / RNA Mechanisms of Triplet Repeat Toxicity**
  (Maurice S. Swanson / Peng Jin / *Laura Ranum* / *Cheryl Wellington*)
- **Silencing RNAs**
  (Greg Hannon / Beverly Davidson / Philip Zamore / *Neil Aronin* / *Nancy Wexler*)
- **Polyglutamine Species, Turnover and Autophagy**
  (David Rubinsztein / Steven Finkbeiner / *Paul Patterson* / *Marcy MacDonald*)
- **Preclinical, Clinical Trials and Development of Biomarkers**
  (Rajiv Ratan / Bernhard Landwehrmeyer / Hindrik Mulder / *Marie-Françoise Chesselet* / *Jenny Morton*)
- **Keynote Plenary Address**
  (Anne B. Young)

CALCIUM SIGNALLING
THE QUEEN'S COLLEGE
OXFORD, UK
JUL 24-29, 2005
COLIN TAYLOR, CHAIR
INDU AMBUDKAR, VICE CHAIR

- **Spatial Organisation of Ca^{2+} Signalling**
  (*Alexei Tepikin* / Mary Kennedy / Gyorgy Hajnoczky / Clara Franzini-Armstrong)
- **Intracellular Ca^{2+} Channels**
  (*Barbara Ehrlich* / Noriaki Ikemoto / Paula da Fonseca / Antony Galione)
- **Ca^{2+} Regulation of Channels**
  (*Susan Hamilton* / Gerda Breitwieser / David Yue / Kevin Foskett)
- **Ca^{2+} Entry**
  (*Jim Putney* / Michael Tymianski / Victoria Bolotina / Anant Parekh)
- **TRP Proteins**
  (*Bernd Nilius* / Michael Caterina / Veit Flockerzi / Richard Sandford)
- **Ca^{2+} and Secretion**
  (*Indu Ambudkar* / Ege Kavalali / Pete Thorn / Shmuel Muallem)
- **Ca^{2+} and Vascular Function**
  (*Mike Berridge* / Mark Nelson / Lother Blatter / Dirk Van Helden)
- **Decoding Ca^{2+} Signals**
  (*Manuela Zaccolo* / Ian Parker / Bob Burgoyne / Winfried Denk)
- **Plenary Lecture**
  (Katsuhiko Mikoshiba)

CANCER MODELS & MECHANISMS
BRYANT UNIVERSITY
SMITHFIELD, RI
JUL 24-29, 2005
PIER PAOLO PANDOLFI, CHAIR
RENE BERNARDS, VICE CHAIR

- **mRNA Translation Control and Cancer**
  (David M. Sabatini / George Thomas / Lewis Cantley / Eric Holland)
- **Nucleolar Network / Ribosome and Cancer**
  (Jacqueline Lees / Jason Weber)
- **Aging, Telomeres and Cancer**
  (Jerry Shay / Elizabeth Blackburn)
- **Microenvironment and Metastasis**
  (Harold Moses / Joan Massagué / William Hahn)
- **Mitotic Cell Cycle Control and Genomic Instability**
  (Prasad Jallepalli / Ashok Venkitaraman / Frederick Alt)
- **Stem Cell and Cancer Stem Cell**
  (George Daley / Michael Clarke / Tetsuo Noda / Len Zon)
- **Cooperative Tumorigenesis**
  (Ron dePinho / Peter Sicinski)
- **Oncogenes, Tumor Suppressors and Modifiers**
  (William Kaelin / René Bernards / Terry Van Dyke)
- **Transcription, Epigenetic Control and Cancer**
  (Maarten Van Lohuizen / Nicholas La Thangue)

CANNABINOID FUNCTION IN THE CNS
BATES COLLEGE
LEWISTON, ME
JUL 17-22, 2005
KEN MACKIE & NEPHI STELLA, CO-CHAIRS

- **Plenary Talk**
  (Gerard Le Fur)
- **Endocannabinoid Synthesis and Inactivations**
  (*Tung Fong* / Daniele Piomelli / Ben Cravatt / Alex Makriyannis)
- **Cannabinoid Receptors and Signal Transduction**
  (*Manuel Guzman* / Deborah Lewis / Tamas Freund / Jean Antoine Girault)
- **Cannabinoids and Neuronal Plasticity I**
  (Wade Regehr / Masanobu Kano / Pablo Castillo / Brad Alger)
- **Cannabinoids and Neuronal Plasticity II**
  (*Jane Sullivan* / Dan Feldman / Sacha Nelson / David Lovinger)
- **Adaptive Changes with Chronic Cannabinoid Use**
  (*Phil Iredale* / Laura Sim-Selley / Olvier Manzoni / Stan Thayer)
- **Food, Aversion, Reward, Sex, and Pain I**
  (*Carl Lupica* / George Kunos / Beat Lutz / Rafael Maldonado)
- **Food, Aversion, Reward, Sex, and Pain II**
  (*George Nomikos* / Don Simone / Aron Lichtman)
- **Interactions with Other Signaling Systems**
  (Andrea Giuffrida / Virginia Pickel / Lakshmi Devi)

CARBOHYDRATES
TILTON SCHOOL
TILTON, NH
JUN 19-24, 2005
GEERT-JAN BOONS, CHAIR
TODD LOWARY &
PENG WANG, CO-VICE CHAIRS

- **New Synthetic Methods for the Synthesis of Complex Oligosaccharides**
  (*Geert-Jan Boons* / Alexei Demchenko / Takashi Takahashi / Andrea Vasella)
- **New Directions in the Target Synthesis of Complex Oligosaccharides and Glycoconjugates**
  (*George Peng Wang* / Jacqueline Gervay-Hague / Zhongwu Guo / Gijs van der Marel)
- **Design, Synthesis and Biological Evaluation of Inhibitors of Carbohydrate Processing Enzymes**
  (*Todd Lowary* / B. Mario Pinto / Vern Schramm)
- **Genomics, Proteomics and the New Era of Glycomics**
  (*Ole Hindsgaul* / Catherine Costello / James Paulson / Nicola Pohl)
- **Carbohydrate Bio-Engineering**
  (Antoni Planas / John Thorson / Stephen Withers)
- **Stuctural Studies of Oligosaccharides and Carbohydrate Binding Proteins**
  (Monica Palcic / Daan van Aalten)
- **Complex Carbohydrates and Microbes**
  (David Bundle / Hung-Wen Liu / Stefan Oscarson / Suzanne Walker)
- **Chemistry and Biochemistry of Glycoproteins and Glycopeptides**
  (Horst Kunz / Yukishige Ito)
- **Glyconanotechnology**
  (Nicolai Bovin / J.P. Kamerling / J. Fraser Stoddart)
- **Recognition of Carbohydrates by Artificial Receptors**
  (Amit Basu / Anthony Davis / Binghe Wang)

CATECHOLAMINES
PROCTOR ACADEMY
ANDOVER, NH
JUL 24-29, 2005
DAVID SULZER, CHAIR
JILL BECKER, VICE CHAIR

- **Behavior and Neurotransmission**
  (*Stephanie Cragg* / Keynote Lecture: Ann Graybiel)
- **Driving Neuronal Activity**
  (*R. Mark Wightman* / Suzanne Haber / Susan Sesack / Peter Redgrave / J. Paul Bolam)
- **Is Dopamine the Substrate for Reward? A Debate**
  (*Jill Becker* / Nora Volkow / P. Read Montague / Kent Berridge / John Salamone)
- **Synaptic Modulation**
  (*D. James Surmeier* / Patricio O'Donnell / John Williams / Jose Bargas)
- **Neurodegenerative Mechanisms**
  (*Serge Przedborski* / William Dauer / Ana Maria Cuervo / Susan Lindquist)
- **Receptors / Second Messengers**
  (*Robert Edwards* / Nigel Bamford / Ivan Diamond / Jeremy Seamans)
- **Workshop: Non-Neurotransmitter Catechols**
  (*Okezie Aruoma* / *Luigi Zecca*)
- **Behavioral Disorders**
  (*Michael Zigmond* / Terry Robinson / Antonello Bonci / Richard Palmiter)
- **Transporters and Presynaptic Regulation**
  (*Nancy Zahniser* / *Jonathan Javitch* / Aurelio Galli / Mark Caron / Annette Fleckenstein / Mark von Zastrow)
- **Workshop: Discussion Group**
  (*Michael Zigmond*)
- **Kopin Award Lectures**
  (to be selected from junior investigators)
- **Integrating Synapses with Behavior**
  (*Anne Etgen* / Regina Carelli / Plenary Lecture: Paul Greengard)

CATCHMENT SCIENCE: INTERACTIONS OF HYDROLOGY, BIOLOGY & GEOCHEMISTRY
COLBY COLLEGE
WATERVILLE, ME
JUL 17-22, 2005
CHRISTINE ALEWELL &
DOUGLAS BURNS, CO-CHAIRS
ELIZABETH BOYER, VICE CHAIR

- **Ecosystem Disturbance**
  (*Peter Groffman* / *Dale Johnson* / Lindsay Rustad / Ralph Boerner / Beate Michalzik / Klement Tockner / Keith Eshleman)
- **Ecosystem Restoration**
  (*David Allan* / *Louise Heathwaite* / Emily Bernhardt / John Quinn / Bob Harris / Joy Zedler / Bob Foy)
- **Field Trip: Acadia National Park Catchments**
  (*Steve Kahl*)
- **Long-Term Monitoring and Experimental Manipulation**
  (*Heleen de Wit* / *Colin Neal* / Christine Goodale / Richard Skeffington / Jill Baron / Peter Dillon / Michel Meybeck)

CELL BIOLOGY OF METALS
BATES COLLEGE
LEWISTON, ME
JUL 3-8, 2005
N ROBINSON & DENNIS WINGE, CO-CHAIRS
ANDREW DANCIS, VICE CHAIR

- **Metal Homeostasis in the Central Nervous System**
  (*Jonathan Gitlin* / John Burn / Leah Harris / Tracey Rouault)
- **Transport of Metal-Complexes**
  (*Jerry Kaplan* / Jon Barasch / Carolyn Philpott / Andy McKie / Mark Fleming)
- **Membrane Metal-Transport**
  (*Nancy Andrews* / Dennis Thiele / Svetlana Lutsenko / Dave Eide)
- **Metal-Responsive Transcription and Signaling**
  (*David Giedroc* / Amanda Bird / Simon Labbe / Simon Whitehall / Walter Schaffner)
- **Post-Transcriptional Metal-Responses**
  (*Betty Leibold* / William Walden / Mick Petris / Glen Andrews)
- **Metals in Organelles**
  (*Val Culotta* / Andy Dancis / Jerry Kaplan / Roland Lill / John Weiss)
- **Intercellular Metal-Homeostasis**
  (*Jim Kushner* / Sophie Vaulont / Nancy Andrews / Tom Ganz)
- **Global Analyses of Metals in Cells**
  (*David Eide* / Tom O'Halloran / Simon Andrews / Mary Lou Guerinot)
- **Metallochaperones and Intracellular Trafficking**
  (*Tom O'Halloran* / Val Culotta / Nigel Robinson / Jonathan Gitlin)

CELL CONTACT & ADHESION
PROCTOR ACADEMY
ANDOVER, NH
JUN 26-JUL 1, 2005
GERALD GRUNWALD, CHAIR
ELISABETTA DEJANA, VICE CHAIR

- **Cadherins in Perspective**
  (Masatoshi Takeichi / Rolf Kemler)
- **Cadherin Genomics and Structural Biology**
  (Deborah Leckband / Rheinhard Gessner / Mitsuhiko Ikura)
- **Cadherins and Cell Signaling**
  (Christopher Chen / Stephen Byers / Barry Gumbiner)
- **Cadherins and Receptor Interaction**
  (Rachel Hazan / Robert Brackenbury / Nikolaos Robakis)
- **Cadherin-Integrin Crosstalk**
  (Jack Lilien / Keith Johnson / Maria Kukuruzinksa)
- **Cadherins and Development I**
  (Walter Birchmeier / James Nelson / Elisabetta Dejana)
- **Cadherins and Cancer**
  (Andrea McClatchey / Al Reynolds / Pamela Cowin)
- **Cadherins and Development II**
  (Christoph Redies / Juliet Daniel / Jeffrey Hardin)
- **Hot Topics**

CELL PROLIFERATION, MOLECULAR & GENETIC BASIS OF
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUN 26-JUL 1, 2005
MICHAEL TYERS, CHAIR
MICHAEL YAFFE, VICE CHAIR

- **Keynotes**
  (Gerard Evan / Steve Elledge)
- **Growth**
  (*Bruce Edgar* / Mike Hall / Morrie Birnbaum / George Thomas / Laura Johnston)
- **Signaling**
  (*Tony Pawson* / Dafna Bar-Sagi / Ben Neel / Helen McNeill)
- **Cell Cycle**
  (*Orna Cohen-Fix* / Wade Harper / Helena Richardson / Matthias Peter / Joan Ruderman)
- **Chromosome Replication and Dynamics**
  (*Titia de Lange* / Vicki Lundblad / John Diffley / Dan Durocher)
- **Checkpoints**
  (*Helen Piwnica-Worms* / Sally Kornbluth / Carol Prives / Susan Lees-Miller / Mike Yaffe)
- **Systematics and Mathematical Modeling**
  (*Fred Cross* / Ravi Iyengar / Rebecca Heald / Mike Snyder)
- **Cancer**
  (*Tak Mak* / Jackie Lees / Jean Wang)
- **Gene Regulation**
  (*Dan Gottschling* / Nick Dyson / Bill Tansey / Ali Shilatifard)

CELLULAR OSMOREGULATION: SENSORS, TRANSDUCERS AND REGULATORS
SALVE REGINA UNIVERSITY
NEWPORT, RI
AUG 7-12, 2005
D. WAYNE BOLEN & IAN BOOTH, CO-CHAIRS
RAINER HEDRICH, VICE CHAIR

- **Cellular Osmoregulation**
  (Maurice Burg / Janet Wood)
- **Stress Response: Sensing and Response Integration**
  (Karlheinz Altendorf / Kazuo Shinozaki / Dietmar Kültz)
- **Cell Volume Regulation**
  (Donald Hilgemann / Paul Blount / Samantha Miller)
- **Macromolecule-Water-Solute Interactions**
  (Valarie Daggett / Gary Pielak / Daniel Harries / Jörg Rösgen)
- **Osmotic Stress: Organism Responses**
  (Richard Morimoto / Volker Müller / Jay Gralla)
- **Signal Transduction and Osmoregulation**
  (Masayori Inouye / Haruo Saito / Julian Schroeder)
- **Aquaporins**
  (Mark Knepper / Christophe Maurel / Andreas Engel)
- **Osmolyte Accumulation and its Impact on Membrane Structure & Stability**
  (Joseph Zacchai / Antoinette Killian / Dennis Dougherty / Fred Sachs)
- **Integrated Function and Osmoregulation**
  (Erhard Bremer / Kevin Strange)

CELLULASES & CELLULOSOMES
PROCTOR ACADEMY
ANDOVER, NH
AUG 7-12, 2005
TUULA TEERI, CHAIR
R. ANTONY WARREN, VICE CHAIR

- **Keynote Lectures**
  (*Rajaj Atalla* / Al Boraston)
- **Cellulose and Cellulose-Binding Modules**
  (*Harry Gilbert* / Paul Knox)
- **Enzyme Producers: Physiology and Genomics**
  (*Joel Cherry* / Merja Penttilä)
- **Enzyme Mechanisms and Engineering**
  (*Marc Cleayssens* / Anu Koivula / Bill Adney)
- **Plant Cellulases**
  (*David Wilson* / Emma Master / Breeanne Urbanowicz)
- **Cellulosomes: Structure, Function and Engineering**
  (*Ed Bayer* / Roy Doi)
- **Enzymatic Processing of Cellulose**
  (*Mike Himmel* / John Tomashek)
- **Other Polysaccharidases**
  (*Colin Mitchinson* / Leila LoLeggio / Maria Hrmova)

CERAMICS, SOLID STATE STUDIES IN
TILTON SCHOOL
TILTON, NH
JUL 17-22, 2005
BRIAN DERBY, CHAIR
JOHN BLENDELL, VICE CHAIR

- **Failure Mechanisms of Si and Devices**
  (Roberto Ballarini / Zhigang Suo)
- **Ferroelectrics: Micromechanics and Fatigue**
  (*Michael Hoffmann* / Jurgen Rödel / Norman Fleck)
- **Nanoindentation of Semiconductor and Ceramic Materials**
  (*Bill Clegg* / Jody Bradby / Robert Cook)
- **Nanocomposites and Nanomechanical Properties of Ceramics**
  (*Raj Bordia* / Giuseppi Pezzotti / Bill Curtin)
- **Fabrication of MEMS Structures**
  (Rob Dorey / Mark Spearing)
- **Novel Fabrication and Synthesis of Inorganic Materials**
  (*John Halloran* / Jennifer Lewis / Rajeesh Naik)
- **Structure/Property Relations in Biological Materials**
  (*Bob McMeeking* / Adrian Mann / Robert Ritchie)
- **Materials in Biomedical Applications**
  (Brian Lawn / Mike Swain)
- **Thursday Night After Dinner Speaker**
  (*Brian Derby* / TBA)

CHEMICAL OCEANOGRAPHY
TILTON SCHOOL
TILTON, NH
AUG 7-12, 2005
CLARE REIMERS, CHAIR
EDWARD BOYLE, VICE CHAIR

- **CO_2 Induced Changes in Ocean Calcification**
  (*Ken Caldeira* / Victoria Fabry / Joanie Kleypas)
- **Land-Ocean Interactions**
  (*Sybil Seitzinger* / Wei-Jun Cai / James Bauer / Thomas Bianchi)
- **Science from Sensors**
  (*Ronnie Glud* / Marylou Tercier-Waeber / Stefan Hulth)
- **Coastal -Open Ocean Connections**
  (*Chen-Tung Arthur Chen* / Denis Gilbert / Burke Hales / Nicolas Gruber)
- **Oceanic Iron: Concentrations and Isotope Compositions**
  (*Robert Sherrell* / Kenneth Johnson / Silke Severmann)
- **Anaerobic Biogeochemistry**
  (*Mary Scranton* / Marcel Kuypers / George Luther / Josef Werne)
- **Crustal Recycling and Fluxes**
  (*Michael Mottl* / Terry Plank / Geoff Wheat)
- **Biomolecules, Speciation and Paleo-Proxies**
  (*Roger François* / James Moffett / Peter Santchi / Gideon Henderson)
- **Reconstructing the Chemistry of Past Oceans**
  (*Ed Boyle* / Rebecca Robinson / Julian Sachs)

CHEMICAL SENSORS & INTERFACIAL DESIGN
THE QUEEN'S COLLEGE
OXFORD, UK
AUG 28-SEP 2, 2005
BORIS MIZAIKOFF, CHAIR
ANTHONY COLEMAN, VICE CHAIR

- **Molecular Recognition**
  (*Boris Mizaikoff* / Jean-Marie Lehn / Christoph Fahrni)
- **Interface Architectures**
  (*Tony Ricco* / Mark Grinstaff / Jerry Atwood / Günther Tovar)
- **Interface Characterization**
  (*Richard Colton* / Andreas Engel / Rachel McKendry)
- **Optical Chemical Sensor Technology**
  (*Reinhard Niessner* / Jerome Faist / Jiri Homola / Karl Unterrainer)
- **News on Electrochemical Sensors**
  (*Ingemar Lundström* / Dave Williams / Mark Lonergan)
- **Nanotechnology in Chemical Sensing**
  (*Andreas Hierlemann* / Phaedon Avouris / James Heath / Reginald Penner)
- **Short Poster Talks**
  (*Anthony Coleman*)
- **New Frontiers**
  (*Elizabeth Hall* / Sylvia Daunert / Kevin Linker / Jeffrey Borenstein)

CHEMISTRY EDUCATION RESEARCH & PRACTICE
CONNECTICUT COLLEGE
NEW LONDON, CT
JUN 26-JUL 1, 2005
STACEY LOWERY BRETZ, CHAIR
CHRISTOPHER BAUER, VICE CHAIR

- **Shaping the Research Agenda in Chemistry Education**
  (*Art Ellis* / Richard Zare / George Bodner)
- **Mentors & Teachers: The Transition States of Our Profession**
  (*Mary Atwater* / Kate Scantlebury / Isiah Warner / Donald Wink)
- **Learning in the Chemistry Laboratory**
  (*Norbert Pienta* / Dawn Rickey / Brian Woodfield)
- **Advances in Measuring Student Learning**
  (*Thomas Holme* / Melanie Cooper / Diane Ebert-May / Angelica Stacy)
- **Learning On-Line: Facilitating Student Understanding**
  (*Jimmy Reeves* / Marcy Hamby Towns / Gregor Novak)
- **Visualization & Language in Learning Chemistry**
  (*Marcia Linn* / Guy Ashkenazi / Roy Tasker / Maria Oliver-Hoyo)
- **The Chemistry of History & The History of Chemistry**
  (*Zafra Lerman* / Pierre Laszlo / Mary Virginia Orna)
- **Student-Centered Learning: The Role of Faculty Development**
  (*Amy Phelps* / Jennifer Lewis / Maureen Scharberg)
- **Disseminating Pedagogical Change: Increasing Our Percent Yield**
  (*Chris Bauer* / Rick Moog / Pratibha Varma-Nelson)

CHROMOSOME DYNAMICS
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUL 31-AUG 5, 2005
ABBY DERNBURG, CHAIR
N. PATRICK HIGGINS, VICE CHAIR

- **Recombination and Genome Plasticity**
  (*David Sherratt* / Nancy Kleckner / John Moran / Susan Lovett)
- **Links Between Chromosome Structure and Function**
  (*John Marko* / John Battista / Nancy Maizels / Nick Cozzarelli / Ling Juan Wu)

- **Evolution of Genomes and Chromosomes**
  (*Matt Meselson* / L. Aravind / Evan Eichler / Fernando Pardo Manuel de Villena)
- **Chromosome Sites with Special Functions**
  (*Julie Cooper* / Arshad Desai / Don Cleveland / Hiro Funabiki / Kelly Dawe / Gary Karpen / George Chaconas)
- **Cell Division and Chromosome Segregation**
  (*Barb Funnell* / Sue Biggins / Kenn Gerdes / Stuart Austin / Jason Swedlow / Daniela Cimini / Rebecca Heald)
- **Meiosis and Sporulation**
  (*Scott Hawley* / Yasushi Hiraoka / Sigal Ben-Yehuda)
- **Organization and Dynamics of Transcriptional Domains**
  (*Barbara Meyer* / Barbara Panning / Dmitry Vassylyev / Shiv Grewal)
- **Condensation and Cohesion**
  (*Tatsuya Hirano* / Rolf Jessberger / Frank Uhlmann)
- **Replication and the Cell Cycle**
  (*Ken Kreuzer* / Alan Grossman / Steve Bell / Susan Forsburg)

CHRONOBIOLOGY
SALVE REGINA UNIVERSITY
NEWPORT, RI
JUL 31-AUG 5, 2005
MARTHA GILLETTE, CHAIR
TILL ROENNEBERG, VICE CHAIR

- **The Whole Cell Clock**
  (*Gene Block* / *Martha Merrow* / Gerald Pollack / Howard Petty / Masayuki Ikeda)
- **Oscillator Networks: Gene and Cell Systems**
  (*Carla Green* / *Paolo Sassone-Corsi* / Deb Bell-Pedersen / Satchin Panda / Shelley Tischkau / Hitoshi Okamura)
- **Oscillator Networks: Cells and Tissues**
  (*Robert Y. Moore* / *Laura Smale* / Erik Herzog / Horacio de la Iglesia / Rae Silver)
- **Entrainment: Molecular Sensors to Behavioral Change**
  (*Russell Foster* / *Susan Golden* / Charalambos Kyriacou / Benjamin Tu / Steven Kay / Michael Rosbash / Till Roenneberg)
- **Clocks, Sleep and Genes**
  (*Joan Hendricks* / *Ketema Paul* / Fred Turek / Giulio Tonini / Paul Shaw)
- **Clocks in Translation**
  (*Ann-Marie Chang* / *Phyllis Zee* / Liz Maywood / Joseph Bass / Marina Antoch / Cheng Chi Lee / Scott Davis)
- **Output Signals and Peripheral Clocks**
  (*Elizabeth Klerman* / *Paul Taghert* / Amita Sehgal / Ruud Buijs / Charles Weitz)
- **Temporal Aspects of Behavioral Integration**
  (*Carolina Escobar* / *David Weaver* / Michaela Hau / Joel Elmquist / Nicholas Mrosovsky / Donald Pfaff / Martin Ralph)
- **Visions of Synthesis**
  (Mick Hastings / *David Welsh* / Patricia Lakin-Thomas / Joseph Takahashi / Michael Menaker)

CLUSTERS, NANOCRYSTALS & NANOSTRUCTURES
CONNECTICUT COLLEGE
NEW LONDON, CT
JUL 31-AUG 5, 2005
MOUNGI BAWENDI &
ORI CHESHNOVSKY, CO-CHAIRS
A. WELFORD CASTLEMAN, JR. &
VICKI COLVIN, CO-VICE CHAIRS

- **Opening Session**
  (Peidong Yang / Daniel Neumark)
- **Electronic Properties**
  (*Paul Alivisatos* / Bernd von Issendorff / Uri Banin / Atsushi Nakajimi)
- **Magnetism**
  (*Lai-Sheng Wang* / Walt de Heer)
- **Optical Properties**
  (*Alex Zunger* / Victor Klimov)
- **Structure**
  (Younan Xia / Martin Jarrold)
- **Nanostructures and Biology**
  (Amit Meller / Christine Keating / Itamar Wilner)
- **Physical Properties**
  (Hellmut Haberland / Uzi Landman)
- **Functional Structures**
  (Hongjie Dai / Eran Rabani / James Heath)
- **Photonics**
  (*Hongkun Park* / Naomi Halas / Yoel Fink)

CO_2 ASSIMILATION IN PLANTS: GENOME TO BIOME
CENTRE PAUL LANGEVIN
AUSSOIS, FRANCE
SEP 11-16, 2005
GEORGE BOWES &
SUSANNE VON CAEMMERER, CO-CHAIRS
ANNE BORLAND &
MICHAEL SALVUCCI, CO-VICE CHAIRS

- **Conference Opening**
  (George Bowes / Susanne von Caemmerer)
- **Photosynthesis in a Higher CO_2 World**
  (*Howard Griffiths* / Gregory Asner / Ulf Riebesell)
- **CO_2 Entry**
  (*James Moroney* / Stephen Maberly / Fiona Woodger / Yuko Hanba)
- **Stomatal Regulation and Metabolism**
  (*Keith Mott* / Sarah Assmann / William Outlaw)
- **Regulation of CO_2-Assimilating Enzymes**
  (*Martin Parry* / Inger Andersson / Archie Portis / Jean Vidal)
- **Chloroplastic Carbon Metabolism**
  (*Christine Raines* / Brigitte Gontero-Meunier / Alison Smith)
- **C_4 and CAM Photosynthesis**
  (*Anne Borland* / Julian Hibberd / Gerald Edwards / John Cushman)
- **Early-Career Scientists and "Hot-Off-The-Press" Short Talks**
  (*Michael Salvucci*)
- **Emerging Technologies**
  (*Hans Bohnert* / Itzhak Kurek / Sacha Baginsky / Sylvie Lalonde / Alisdair Fernie)
- **After-Banquet Featured Speaker**
  (*Susanne von Caemmerer* / Mark Stitt)

COASTAL OCEAN CIRCULATION
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUN 5-10, 2005
STEVEN LENTZ, CHAIR
JAMES O'DONNELL, VICE CHAIR

- **Dispersal of Terrestrial Runoff**
  (*James O'Donnell* / John H. Simpson / Robert Chant)
- **Shelf Circulation and Shelf Edge Processes**
  (*Robert Beardsley* / John S. Allen / Glen Gawarkiewicz / Edward Dever)
- **High Latitude Shelf Processes**
  (*Tom Weingartner* / Robert Pickart / John Klinck)
- **Physical Influences on Coastal Ecosystems**
  (*Richard Signell* / Jonathan Sharples / Andy Visser / David Townsend)
- **Nearshore Processes**
  (*Gail Kineke* / Peter Traykovski / Tuba Ozkan-Haller)
- **Estuarine and (Very) Shallow Water Dynamics**
  (*Parker MacCready* / James Lerczak / Mark Stacey / Heidi Nepf)
- **Coastal Interactions with the Atmosphere**
  (*Roger Samelson* / James Edson / John Bane)
- **Vertical Mixing**
  (*Tom Rippeth* / Jonathan Nash / Jennifer MacKinnon / Eric D'Asaro)
- **Scientific Insights from Coastal Observatories**
  (*Steven Lentz* / Jack Barth / Clinton Winant)

COATINGS & FILMS
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUL 10-15, 2005
DOUGLAS WICKS, CHAIR
WILLIAM SIMONSICK &
JUDITH STEIN, CO-VICE CHAIRS

No information available at press time. Please check the GRC web site for details.

COLLAGEN
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUL 24-29, 2005
PETER BRUCKNER, CHAIR
DAVID BIRK, VICE CHAIR

- **New Collagens**
  (*Florence Ruggiero*)
- **Expression, Biosynthesis and Regulation of Collagens**
  (*Johanna Myllyharju*)
- **Protease Processing of Matrix**
  (*Suneel Apte*)
- **Supramolecular Matrix Assemblies**
  (*Paul Bornstein*)
- **Cell Adhesion to Matrix - Cryptic Adhesion**
  (*Johannes Eble*)
- **Collagen Diseases**
  (*Leena Ala-Kokko*)
- **Gene Therapy and Regenerative Medicine**
  (*Leena Bruckner-Tuderman*)
- **Invertebrate and Vertebrate Animal Models**
  (*Kathy Cheah*)
- **Hot Topics in the Field**

COMBINATORIAL & HIGH THROUGHPUT MATERIALS SCIENCE
THE QUEEN'S COLLEGE
OXFORD, UK
AUG 14-19, 2005
JAMES CAWSE &
WILHELM MAIER, CO-CHAIRS
DAVID ROTHMAN &
ULRICH SCHUBERT, CO-VICE CHAIRS

- **How to Handle Diversity - Biomaterials and Enzymes**
  (*Oleg Kolosov* / Manfred T. Reetz / Bruce Eaton)
- **Theoretical Aspects of Materials Discovery**
  (*James Cawse* / Dimitris Agrafiotis / Francois Gilardoni / Mikk Lippmaa)
- **Discovery of Knowledge**
  (*Claude Mirodatos* / Ferdi Schueth / Fred Hamprecht)
- **Materials Development**
  (*Eric Amis* / Y. Matsumoto / K.-S. Sohn / Oden Warren)
- **Search for Novel Materials**
  (*Ichiro Takeuchi* / Charles Olk / Seong Woo)
- **Industrial Experiences with HTE**
  (*William Flanagan* / Jennifer Holmgren / Thomas Brinz / Victor Adamian / Katharine Allen)
- **Discovery and Optimization of Heterogeneous Catalysts**
  (*Dirk Demuth* / Pierre Jacobs / Peter Claus)
- **Discovery and Optimization of Polymers**
  (*Ulrich Schubert* / Rolf Muelhaupt / Mark Bradley / Kathryn Beers / Chris Stafford)
- **Discovery and Applications of Homogeneous Catalysts**
  (*M.G. Finn* / Olivier Lavastre / Albrecht Berkessel)

COMBINATORIAL CHEMISTRY
PROCTOR ACADEMY
ANDOVER, NH
AUG 21-26, 2005
SAMUEL GERRITZ, CHAIR
R. KIP GUY, VICE CHAIR

- **Target Class Libraries**
  (*Christine Brotherton-Pleiss* / Hartmuth Kolb / David Drewry / Jon Ellman)
- **Case Studies I**
  (*Daryl Sauer* / Brian McKittrick / Rachael Hunter / Guy Breitenbucher / Craig Lindsley / Stefan Warner)
- **Library Synthesis Capacity: Rent or Buy?**
  (*John Porco* / James Connelly / Libing Yu / Bill Coates)
- **Libraries Based on Natural Products**
  (*Sam Gerritz* / Takashi Takahashi / Gunda Georg / Ganesan / Annaliese Franz / Scott Schaus)
- **CMLD Overview**
  (*John Schwab* / John Porco / Stefan Werner / Jeff Aube / Harvard)
- **Synthesis and Screening of Biopolymers**
  (*Shelli McAlpine* / Peter Seeberger / Laura Kiessling / Alice Ting / Akira Kawamura)
- **Novel Solid- and Solution-Phase Synthetic Methods**
  (*John Quinn* / Rolf Breinbauer / Eric Jacobson / Mark Bradley)
- **Case Studies II**
  (*Richard Austin* / Jeremy Green / Anna Pendri / Christopher Hulme / Ivan Lorkovic)
- **Chemical Genetics**
  (*Kip Guy* / Anna Mapp / Young-Tae Chang / Tom Kodadek)

COMPUTER AIDED DRUG DESIGN
TILTON SCHOOL
TILTON, NH
JUL 31-AUG 5, 2005
PETER JURS, CHAIR
RICHARD LEWIS, VICE CHAIR

- **Scoring Docking Poses**
  (*Michal Vieth* / Chris Murray / Arthur Doweyko)
- **Cheminformatics I**
  (*Mik Lajiness* / Gavin Harper / Val Gillet / Mark Mackey)
- **Cheminformatics II**
  (*Dan Ortwine* / Bob Clark)
- **Energetics of Molecular Recognition**
  (*Andy Good* / Ernersto Frieire / Celia Schiffer / Adrian Elcock)
- **Modelling GPCRS**
  (*Antonia do Amaral* / Caterina Bissantz)
- **Protein Modelling**
  (*Brian Shoichet* / Holger Gohlke / Andrej Sali)
- **State of the Nation**
  (*Tudor Oprea* / Garland Marshall)
- **New Technologies**
  (*Tom Fox* / Jon Mason / Nicolas Froloff)
- **Case Studies**
  (*Bill Egan* / Mike Sabio)

CONDENSED MATTER PHYSICS
CONNECTICUT COLLEGE
NEW LONDON, CT
JUN 19-24, 2005
PAUL MCEUEN, CHAIR
ROBERT MAGERLE, VICE CHAIR

- **Biology Meets Materials**
  (Joanna Aizenberg)
- **Colloids as Molecules, Liquids, and Solids**
  (David Weitz / David Pine)
- **Membrane Interfaces and Networks**
  (Jay Groves / Owe Orwar)
- **Novel Manipulation and Imaging Techniques**
  (Robert Westervelt / Lois Pollack)
- **Nano Meets Bio**
  (Serge Lemay / Luke Lee / Michael Roukes)
- **Vortices**
  (David Nelson / Cynthia Olson Reichardt)
- **Synthesis**
  (Rustem Ismagilov / Tom Mallouk)
- **Future of Condensed Matter Physics**
  (Sid Nagel)

DETECTING ILLICIT SUBSTANCES: EXPLOSIVES & DRUGS
LES DIABLERETS CONFERENCE CENTER
LES DIABLERETS, SWITZERLAND
AUG 28-SEP 2, 2005
RICHARD LAREAU & JEANNE LIN, CO-CHAIRS

- **Policy Discussion: Privacy Versus Security**
- **Trace Detection Technologies - I: Sampling and Sensing & Detection**
- **Olfactory and Detection R&D**
- **Bulk Technologies - I**
- **Emerging Technologies**
- **Special Session: Near and Far-Range Standoff Detection**
- **Bulk Technologies - II**
- **Trace Technologies - II: Detection**
- **Challenges for the Future: An International Perspective**

DEVELOPMENTAL BIOLOGY
PROCTOR ACADEMY
ANDOVER, NH
JUN 19-24, 2005
RICHARD HARLAND, CHAIR
STEPHEN COHEN, VICE CHAIR

- **Asymmetry and Cell Migration**
  (*Richard Harland* / Chris Doe / Rueyling Lin / Ruth Lehmann / Chris Wylie / Janet Rossant)
- **Genomic and Genetic Approaches (I)**
  (*Ruth Lehmann* / Julie Ahringer / Norbert Perrimon / Makoto Furutani-Seiki / Ron Plasterk / Victor Ambros)
- **Evolution, Morphogens and Modeling**
  (*Julie Ahringer* / Mike Levine / Naama Barkai / Robb Krumlauf / Nipam Patel)
- **Growth and Patterning**
  (*Janet Heasman* / Laura Johnson / Matt Freeman / Joel Rothman / David Wilkinson / Detlef Arendt)
- **Cell Migration and Boundary Formation**
  (*Elizabeth Robertson* / Marianne Bronner-Fraser / Pernille Rørth / Olivier Pourquie / Ray Keller)
- **Signaling and Pattern Formation**
  (*Pernille Rørth* / Roel Nusse / Christoph Niehrs / Phil Soriano / Arthur Lander / Gail Martin / Alex Schier)
- **Growth and Pattern Formation (II)**
  (*Marianne Bronner-Fraser* / Liz Robertson / Lilianna Solnica-Krezel / Ernst Hafen / Iswar Hariharan)
- **Signaling and Morphogenesis During Organogenesis**
  (*Laura Johnson* / Janet Heasman / Andy McMahon / Scott Fraser / Richard Harland / Doug Melton)
- **Growth and Pattern (III)**
  (*Stephen Cohen* / Gary Ruvkun / Cliff Tabin / Stephen Cohen)

DRUG METABOLISM
HOLDERNESS SCHOOL
PLYMOUTH, NH
JUL 10-15, 2005
LARRY WIENKERS, CHAIR
LESLIE BENET, VICE CHAIR

- **Advances in the Basic Knowledge of Cytochrome P450 Towards the Understanding of Drug Metabolism: Part 1 - Structure/ Function**
  (*Stephen G. Sligar* / Michael R. Waterman / Ilme Schlichting / Eric F. Johnson / Richard N. Armstrong)
- **Advances in the Basic Knowledge of Cytochrome P450 Towards the Understanding of Drug Metabolism: Part 2 - Oxidation Reactions**
  (*William M. Atkins* / Jeffrey P. Jones / Sason Shaik / Stephen G. Sligar)

- **Drug Metabolism-Consequences to Toxicity / Pharmacology**
  (*Judy Bolton* / Jim Fishbein / Natalia Tretyakova / Gregory R.J. Thatcher / John M. Essigmann)
- **Expression and Tissue Distribution of Drug Metabolizing Enzymes**
  (*Jeffery C. Stevens* / J. Steven Leeder / Xinxin Ding / Ronald N. Hines)
- **Integrating Preclinical Information to Predict Human Drug Disposition**
  (*Timothy S. Tracy* / Leslie Z. Benet / R. Scott Obach / Mary Paine / J. Brian Houston)
- **Innovative Clinical Approaches in Underwriting Disposition Factors in Early Drug Development**
  (*Paul G. Pearson* / Scott Patterson / Richard Hargreaves)
- **Novel Technology Approaches to Applied to Drug Discovery and Development Drug Metabolism**
  (*Kenneth R. Korzekwa* / Daniel E. Murnick / William M. Atkins / Catherine Booth-Genthe)

DYNAMICS AT SURFACES
PROCTOR ACADEMY
ANDOVER, NH
AUG 14-19, 2005
GILBERT NATHANSON, CHAIR
BRET JACKSON, VICE CHAIR

- **State-to-State Dynamics**
  (*Rainer Beck* / Arthur Utz / Stephen Holloway)
- **Catalysis**
  (*Franz Geiger* / David King / Berit Hinnemann / Bengt Kasemo)
- **Liquid-Liquid Interfaces**
  (*Heather Allen* / Robert Walker / Ilan Benjamin)
- **Dynamics of Adsorption**
  (*Eckart Hasselbrink* / Axel Gross / Mary Jane Shultz / John Morris)
- **Gas-Liquid Interfaces**
  (*Akihiro Morita* / Pavel Jungwirth / Gunther Andersson)
- **Motions at Surfaces**
  (*Bret Jackson* / Jascha Repp / Ellen Williams / William Allison)
- **Electron Dynamics**
  (*Jane Hinch* / Martin Wolf / Giacinto Scoles)
- **Reactions at Surfaces**
  (*Bruce Garrett* / Sylvia Ceyer / Matthias Scheffler / Robert Hamers)
- **Young Investigator Presentations**
  (*Greg Sitz*)

ELASTIN & ELASTIC FIBERS
KIMBALL UNION ACADEMY
MERIDEN, NH
JUL 31-AUG 5, 2005
CAY KIELTY, CHAIR
ELAINE DAVIS, VICE CHAIR

- **Structures of Elastic Fiber Molecules**
  (*Penny Handford* / *Fred Keeley* / John Parkinson / Kristin Kumashiro / Tony Tamburro / Clair Baldock / Sacha Jensen)
- **Microfibril and Elastic Fiber Assembly**
  (*Brenda Rongish* / *Dieter Reinhardt* / Cay Kielty / Tom Wight / Andras Czirok / Sarah Dallas / Jessica Wagenseil)
- **Elastic Tissue Development**
  (*Robert Mecham* / *Charles Little* / Evan Zamir / Eiichi Hirano)
- **Lysyl Oxidases**
  (*Katalin Csiszar* / *Robert Rucker* / Herb Kagan / Philip Trackman / Pascal Sommer / Tiansen Li / Ian Hornstra)
- **Elastic Fiber Molecules - Knock-Out Models**
  (*Elaine Davis* / *Michael Shipley* / Giorgio Bressan / Jouni Uitto / Luca Carta)
- **Heritable Elastic Fiber Diseases**
  (*Zsolt Urban* / *Dianna Milewicz* / Geert Mortier / Edwin Stone / András Váradi / Ivonne Ronchetti)
- **Elastic Fibers and Growth Factors**
  (*Lynn Sakai* / *Dan Rifkin* / Vesna Todorovic / Gerhardt Sengle / Katri Koli / Bart Loeys)
- **Degenerative Elastic Fiber Disorders**
  (*Bill Parks* / *Steve Shapiro* / Marlene Rabinovitch / Robert Thompson / Steve Shapiro / Charles Boyd / Richard Pierce)
- **Elastic Fiber Engineering and Biomaterials**
  (*Tony Weiss* / *Ivan Vesely* / Fred Keeley / Alex Seifalian / Richard Black)

ELASTOMERS, NETWORKS & GELS
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUL 17-22, 2005
GREGORY MCKENNA, CHAIR
H. HENNING WINTER, VICE CHAIR

- **Novel Systems**
  (*Andy Tsou* / Kenji Urayama / Tomiki Ikeda / B.R. Ratna)
- **Biomolecular Networks and Biological Systems (1)**
  (*John Enns* / Lynden Archer / Julie Kornfield / Nathan Ravi / Ferenc Horkay)
- **Poster Sessions**
  (*Henning Winter* / *Manika Varma-Nair*)
- **Physics of Gels**
  (*Athene Donald* / Bela Joos / Murugappan Muthukumar / Christian Holm)
- **Theory and Simulation**
  (*Michael Rubinstein* / Sergey Panyukov / John Curro / Alex Levine / Fernando Escobedo)
- **Biomolecular Networks and Biological Systems (2)**
  (*Marie-France Vallat* / David Mooney / Dennis Discher / Jian Ping Gong)
- **Pot Pourri (1)**
  (*Judit Puskas* / Annelise Barron / Jan Genzer / Darrin Pochan)
- **Pot Pourri (2)**
  (*Lynn Loo* / Tatiana Budtova / Chris Robertson / Jennifer West)
- **Evolving Networks**
  (*Francois Lequeux* / Luis Dorfmann / Alan Wineman / Will Mars)
- **Filled Systems and Nanocomposites**
  (*Chris White* / Jose Kenny / Helene Montes / Alan Gent)
- **Special Topic**
  (*Greg McKenna* / Gerald Pollack)

ELECTRONIC MATERIALS, CHEMISTRY OF
CONNECTICUT COLLEGE
NEW LONDON, CT
JUL 17-22, 2005
CHERIE KAGAN &
THOMAS MALLOUK, CO-CHAIRS
CHRISTOPHER CHIDSEY &
GARY TAYLOR, CO-VICE CHAIRS

- **Nanocrystal Materials and Devices**
  (Cathy Murphy / Alfons von Blaarden / Yang Yang)
- **Molecular Junctions**
  (Stuart Lindsay / James Kushmerick / Theresa Mayer / Mark Ratner)
- **Frontiers of Electronic and Magnetic Materials / Extraordinary Posters**
  (Harry Atwater)
- **Organic Devices**
  (Paul Barbara / George Malliaras / Henning Sirringhaus / Janos Veres)
- **Nanoelectronics**
  (Mildred Dresselhaus / Joerg Appenzeller / Lars Samuelson / Georg Duesberg)
- **Biomolecular and Supramolecular Electronics**
  (Samuel Stupp / Amy Blum)
- **Semiconductor Materials, Growth and Devices**
  (Judy Hoyt / Jeff Calvert / Veena Misra / Robert Hicks)
- **Polymeric Materials and Patterning**
  (Christopher Ober / Charles Black / Ralph Dammel)
- **Electronics at Interfaces**
  (Ellen Williams / Eric Garfunkel)

ENGINEERING SCIENCES FOR SPACE EXPLORATION
LES DIABLERETS CONFERENCE CENTER
LES DIABLERETS, SWITZERLAND
AUG 21-26, 2005
G. PAUL NEITZEL, CHAIR
JAMES WARREN, VICE CHAIR

- **Granular Media & Particulate Flow I**
  (*Pierre Evesque* / James Jenkins / Christine Hrenya)
- **Granular Media & Particulate Flow II**
  (*Iwan Alexander* / Masami Nakagawa / Jeffrey Morris / Michel Louge)
- **Multiphase Flow**
  (*Vemuri Balakotaiah* / Mark McCready / Andrea Prosperetti)
- **Multiphase Heat Transfer**
  (*Alcina Mendes* / Cila Herman / Kambiz Vafai / Johannes Straub)
- **Nanofluidics**
  (*Marc Smith* / Flavio Noca / Joel Plawsky)
- **Biological Life Support Systems**
  (*Jitendra Joshi* / Katherine Banks / Andrew Jackson / Dani Or)
- **Environmental Monitoring**
  (*Gary Sayler* / Marc Porter / James Lambert)
- **Applied Microfluidics / Heat Transfer**
  (*Richard Lueptow* / Ward TeGrotenhuis / Mark Weislogel / Jamal Yagoobi)
- **Cryogenics**
  (*Daniel Beysens* / Louis Salerno / Mohammad Kassemi)

ENZYMES, COENZYMES & METABOLIC PATHWAYS
KIMBALL UNION ACADEMY
MERIDEN, NH
JUL 17-22, 2005
EUGENE MUELLER &
MARTIN TANNER, CO-CHAIRS
SUSAN MILLER &
JOHN RICHARD, CO-VICE CHAIRS

- **Emerging Systems and Methods**
  (*Francisco Wilson* / Wilfred A. van der Donk / Karen S. Anderson / Homme W. Hellinga / Joseph A. Krzycki)
- **Enzyme Mechanisms I**
  (Nigel G.J. Richards / Debra Dunaway-Mariano / Peter J. Tonge / Stewart L. Fisher)
- **RNA: Enzyme and Substrate**
  (Barry S. Cooperman / Tamara L. Hendrickson / Dirk Iwata-Reuyl)
- **Carbohydrates**
  (Gideon J. Davies / Paul J. Berti / James H. Naismith / Vern L. Schramm)
- **Cofactors**
  (Adelbert Bacher / Ruma V. Bannerjee / Holly R. Ellis / Dennis R. Dean)
- **Enzyme Mechanisms II**
  (Michael A. Marletta / Johannes Rudolph / Kenny K. Wong)
- **Frontiers in Enzymology**
  (Perry A. Frey / Rowena G. Matthews)

EPIGENETICS
HOLDERNESS SCHOOL
PLYMOUTH, NH
AUG 7-12, 2005
JUDITH BENDER &
CHAO-TING WU, CO-CHAIRS
ANNE FERGUSON-SMITH &
STEVEN JACOBSEN, CO-VICE CHAIRS

- **Inheritance: Remembrance of Things Past**
  (Marisa Bartolomei / Vicki Chandler / Robert Pruitt / James Sherley / others)
- **Crosstalk: Development and Defense**
  (David Baulcombe / Jeannie Lee / Robert Metzenberg / Benjamin Normark / Amy Pasquinelli / Carmen Sapienza / others)
- **Change and the Environment**
  (Jean Finnegan / Eric Richards / Suzanne Rutherford / Emma Whitelaw / others)
- **Crosstalk: Getting the Message Across**
  (Denise Barlow / James Birchler / William Kelly / Marjori Matzke / Craig Pikaard / Wolf Reik / others)
- **One Scoop or Two: Dosage and Gene Regulation**
  (Ueli Grossniklaus / Edith Heard / Jennifer Marshall-Graves / Ortrun Mittelsten Scheid / others)
- **Switching: Decisions, Decisions, Decisions**
  (Steve Henikoff / Rudolf Jaenisch / Amar Klar / Erika Matunis / Barbara Panning / Jasper Rine / others)
- **Everything in its Place: Genome Organization**
  (Robin Allshire / Anne Ferguson-Smith / Thomas Jenuwein / Robert Martienssen / others)
- **Keeping the Peace**
  (Timothy Bestor / Sarah Elgin / Steve Jacobsen / Eric Selker / others)
- **Looking Forward**
  (special consideration will be given throughout all sessions to include new topics, puzzling observations, and mysterious phenomena)

EVOLUTIONARY & ECOLOGICAL FUNCTIONAL GENOMICS
THE QUEEN'S COLLEGE
OXFORD, UK
JUL 31-AUG 5, 2005
THOMAS MITCHELL-OLDS, CHAIR
GREG WRAY, VICE CHAIR

- **Current Progress and Future Directions**
  (*Thomas Mitchell-Olds* / Enrico Coen / Trudy MacKay / Paul Brakefield)
- **Genomes and the Environment**
  (*Joy Bergelson* / Laurent Keller / Katie Peichel / Tom Whittham)
- **Genomics of Complex Traits**
  (*Trudy MacKay* / Scott Edwards / Jeff Feder / Bill Jeffery)
- **Transcription and Evolution**
  (*Dan Hartl* / Justin Fay / Carol Lee / Patricia Wittkopp)
- **Natural Selection and the Genome**
  (*Joe Thornton* / Dan Hartl / Henrik Kaessman / Sue Wessler)
- **Evolutionary Ecology of Microbial Genomes**
  (*Anthony Dean* / Jennifer Hughes / Martin Parniske / Derek Smith)
- **Plant Model Systems in EEFG**
  (*Enrico Coen* / Joy Bergelson / Caroline Dean / Cynthia Weinig)
- **Evolution of Protein Function**
  (*Sue Wessler* / Anthony Dean / Joe Thornton / Mariana Wolfner)

EXCITATORY AMINO ACIDS & BRAIN FUNCTION
CENTRE PAUL LANGEVIN
AUSSOIS, FRANCE
SEP 4-9, 2005
MORGAN SHENG, CHAIR
ROBERT MALENKA, VICE CHAIR

- **Keynote Lectures**
  (Susumu Tonegawa / Rob Malenka)
- **High Resolution Studies of Glutamate Receptors**
  (Daniel Choquet / Haruo Kasai / Eric Gouaux / Kristen Harris / Guosong Liu / Scott Thompson)
- **Controversies in AMPA Receptor Trafficking**
  (Rick Huganir / Roberto Malinow / David Bredt / Ed Ziff)
- **Synaptic Plasticity Mechanisms**
  (Graham Collingridge / Roger Nicoll / Gina Turrigiano / Yu Tian Wang / Julie Kauer / John Isaac)
- **Genetic Approaches to Glutamate Receptors and Brain Function I**
  (Masayoshi Mishina / Steve Heinemann / Peter Seeburg / Joe Z. Tsien)
- **Experience-Dependent Plasticity In Vivo**
  (Mark Bear / Takao Hensch / Holly Cline / Dan Madison / Dan Feldman)
- **Genetic Approaches to Glutamate Receptors and Brain Function II**
  (Jeremy Henley / Josh Kaplan / Villu Maricq / Stefan J. Sigrist)
- **Development and Turnover of Excitatory Synapses**
  (Mike Ehlers / Kim McAllister / Ann Marie Craig / Monica di Luca / Gary Westbrook)
- **Glutamate Receptor Modulation**
  (Juan Lerma / Suzanne Zukin / Christoph Mulle / Laurent Fagni)

FERTILIZATION & ACTIVATION OF DEVELOPMENT
HOLDERNESS SCHOOL
PLYMOUTH, NH
JUL 17-22, 2005
RICHARD CARDULLO, CHAIR
GEORGE GERTON, VICE CHAIR

- **Bioinformatic, Genomic, and Proteomic Approaches to Fertilization and Development**
  (Anna Neill / Tim Kroft)
- **Sperm Motility and Chemotaxis**
  (*George Witman* / Winfield Sale / Chris Wood / Marc Spehr)
- **Gamete Maturation**
  (*Laurinda Jaffe* / Lisa Mehlman / Khaled Machaca)
- **Functional Membrane Domains in Gametes**
  (*George Gerton* / Alexander Travis / Bart Gadella / Nonunj Tanphaichitr)
- **Molecular Aspects of Sperm-Egg Interaction**
  (*Catherine Thaler* / Daniel Hardy / Masaru Okabe)
- **Signal Transduction in Sperm**
  (*Harvey Florman* / Melissa Jungnickel / Benjamin Kaupp / Dejian Ren)
- **Keynote Lecture**
  (Anne McLaren)
- **Signaling Events During Egg Activation**
  (*Jay Baltz* / Guillaume Halet / Rafael Fissore / Janice Evans)
- **Gene Activation During Early Development**
  (*Kathleen Foltz* / Michelle Roux / Fabio Piano / Michael Stitzel)

FLORAL & VEGETATIVE VOLATILES
THE QUEEN'S COLLEGE
OXFORD, UK
SEP 11-16, 2005
JOHN PICKETT & BRIAN SMITH, CO-CHAIRS
WITTKO FRANCKE &
ERAN PICHERSKY, CO-VICE CHAIRS

- **Analytical Techniques for Analysis of Floral and Vegetative Volatiles**
- **Neural Representation of Odors and the Statistics of Natural Odor Scenes**
- **Biology of Defensive Volatiles**
- **Biology of Attractant Volatiles**
- **Natural History of Volatiles**

FREE RADICAL REACTIONS
HOLDERNESS SCHOOL
PLYMOUTH, NH
JUL 3-8, 2005
MICHAEL SCHMITTEL, CHAIR
JAMES TANKO, VICE CHAIR

- **Radicals in Biology I+II**
  (*Martin Newcomb* / Joan Broderick / Joseph Jarrett / Christian Schöneich / Chrys Chatgilialoglu)
- **Synthetic Applications of Nitroxides**
  (*Armido Studer* / Rebecca Braslau / Paul Tordo)
- **Radical Ions / Radicals and Metals I+II**
  (*Joseph Dinnocenzo* / Mitsuo Sawamoto / James Franz / Shunichi Fukuzumi / Jack Norton)
- **Mechanisms in Radical Chemistry**
  (*James Tanko* / Carl Schiesser / Georg Gescheidt / John Walton)
- **Radicals in Synthesis**
  (*Chrys Chatgilialoglu* / Armido Studer / David Crich / Philippe Renaud)
- **Radicals and DNA**
  (*Michael Schmittel* / Thomas Carell / Shana Kelley)
- **Cheves Walling Lecture**
  (*David Crich* / Bernd Giese)

FUEL CELLS
BRYANT UNIVERSITY
SMITHFIELD, RI
JUL 17-22, 2005
KLAUS-DIETER KREUER &
THOMAS SCHMIDT, CO-CHAIRS
BRIAN BENICEWICZ &
JEREMY MEYERS, CO-VICE CHAIRS

- **The Frontier**
  (D. Wilkinson / D. Thompsett)
- **Membranes**
  (S. Paddison / B. Pivovar / M. Liu)
- **Beyond the Membrane**
  (J. Newman / W. Petitt)
- **Durability/Degradation/Diagnostics**
  (R. Balliet / E. Roduner / M. Inabe / H.R. Kunz)
- **(Electro)Catalysis: From Ensembles to Single Atoms**
  (G. Lindbergh / M. Koper / E. Savinova / U. Heiz / P. Serp)
- **PEM Fuel Cells: The Only Choice?**
  (W. Schnurnberger / S. Haile)
- **Are We on the Right Track?**
  (U. Wagner)

GENETIC TOXICOLOGY
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUL 31-AUG 5, 2005
BENNETT VAN HOUTEN, CHAIR
ANTONY CARR, VICE CHAIR

- **Keynote Talks**
  (*Bennett Van Houten* / Samuel Wilson / Peggy Jeggo / Marc Vidal)
- **Genotoxicity of Endogenous Lesions and BER**
  (*Larry Marnett* / Sankar Mitra / John Essignman / Akira Yasui / Leona Samson)
- **New Developments in Excision Repair**
  (*Jean-Marc Egly* / Caroline Kisker / Kioji Tanaka / Cilla Cooper)
- **Responses to Replication Blockage**
  (*Tom Kunkel* / John Diffley / Alan Lehmann / Jean Wang)
- **Double Black Diamonds of Repair: DNA Cross-Links and Protein-DNA Cross-Links**
  (*Stephen Lloyd* / Peter McHugh / Laura Neiderhofer/ Alan D. Andrea)
- **Damage Inducible Checkpoints and Signaling**
  (*Tony Carr* / Steve Elledge / Rodney Rothstein / Yossi Shilo)
- **DNA Damage In Vivo and Somatic Mutation In Vivo**
  (*Andrew Collins* / Bernd Eppe / Vern Walker / Shinya Shibutani)
- **Mitochondria: The Other Site of DNA Damage**
  (*William Copeland* / Susan Ledoux / Ute Moll / Takehiko Shibata)
- **Metals as Co-Carcinogens**
  (*David Wilson* / Andy Kligerman / Anatoly Zhitkovich / Jim Imlay)

GLOBAL ASPECTS OF TECHNOLOGY TRANSFER: BIOTECHNOLOGY
THE QUEEN'S COLLEGE
OXFORD, UK
SEP 4-9, 2005
JOHN KILAMA, CHAIR
RICHARD MAHONEY, VICE CHAIR

- **Defining Technology Transfer: Past, Present and Future**
  (*Maria Freire* / John Kilama / Lita Nelsen / Robert Mallett)
- **Biotechnology Policy and Legislation**
  (*Robert Horsch* / George Atkinson / Katsuya Tamai)
- **Biotechnology Regulatory and Judiciary**
  (Pauline Newman / Philip Grubb / Tony Bates)
- **Technology Transfer in Developing Countries: Case Studies and Policy Analysis**
  (*Hanna Kettler* / Tony Heher / Joachim Oehler / Zhu Chen / Victoria Hale)
- **University/Industry/Government Partnerships**
  (*Roy Widdus* / Jerry Keusch / Jim K. Muhwezi / Mikyung Yun / Susan Finston)
- **Global Technology Transfer: Its Impact on Product Development, Trade, Litigation and Bio-Security**
  (*Harvey Bale* / Norman Neureiter / J.D. Richard Wilder / Jorge Amigo Castañeda)
- **Global Business Market in Biotechnology**
  (*Calos Morel* / Eric Poincelet / Wendy Taylor)
- **New Paradigms for Assessing Technology Transfer**
  (*Keith Maskus* / Rafael Rangel-Alda / Marcel D. Mongeon / Charles A. Gardner / Usha R. Balakrishnan)
- **Impact of Technology Transfer on Diagnostics and Pharmaceutical Development**
  (*Robert Ridley* / Gail Cassell / Nelson Sewankambo / Kulvinder Singh Saini / Rich Mahoney)

HETEROCYCLIC COMPOUNDS
SALVE REGINA UNIVERSITY
NEWPORT, RI
JUL 3-8, 2005
HUW DAVIES, CHAIR
JOHN MACOR, VICE CHAIR

- **New Synthetic Strategies**
  (*John Macor* / Amir Hoveyda / Barry Trost)
- **Synthesis of Heterocycles**
  (Tom Hoye / Joel Hawkins / Robert Batey)
- **Heterocyclic Transformations**
  (*Michael Van Nieuwehnze* / Vladimir Gevorgyan)
- **Biologically Significant Heterocycles**
  (*Karl Scheidt* / Greg Wayne / Louis Jungheim / Melanie Sanford / Dennis Wright)
- **Assembly of Complex Heterocycles**
  (*Chulbom Lee* / Donald Hertzog / Timothy Guzi / Mark Lautens)
- **New Applications of Heterocycles**
  (Helen Blackwell / Jieping Zhu / Jaume Balsells-Padros / Kevin Burgess / Christine Tarby)
- **Synthesis and Catalysis**
  (Carsten Bolm / Jeffrey Johnston)
- **Organometallic and Heterocyclic Synthesis**
  (*Mikund Sibi* / Timothy Donohoe / Wendy Young / Richard Larock)
- **Heterocycles and Total Synthesis**
  (*E.C. Taylor* / Dean Toste / Erik Sorensen)

HIGH TEMPERATURE CORROSION
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUL 24-29, 2005
BRIAN GLEESON, CHAIR
W QUADAKKERS, VICE CHAIR

No information available at press time. Please check the GRC web site for details.

HORMONE ACTION IN DEVELOPMENT & CANCER
MOUNT HOLYOKE COLLEGE
SOUTH HADLEY, MA
JUL 10-15, 2005
MAARTEN BOSLAND, DARCY KELLEY &
CHERYL WALKER, CO-CHAIRS
SHIUAN CHEN &
PAOLO SASSONE-CORSI, CO-VICE CHAIRS

- **Non-Steroid Hormone Nuclear Receptor Mechanisms**
  (*Paolo Sassone-Corsi* / Bruce Spiegelman / David Moore)
- **Critical Transitions in Development**
  (*Donald Brown* / *Jim Truman* / Kevin White / Alex Schrieber / Cheryl Sisk)
- **Epigenetic Mechanisms in Hormone Action and Imprinting**
  (*John McLachlan* / Gail Prins & Shuk-Mei Ho / David Jarrard / Ken Nephew)
- **Prevention and Therapy of Hormone-Related Cancer**
  (*Ronald Ross*)
- **Animal Models of Hormone Related Cancer and Other Disease**
  (*Jim Shull* / Myles Brown / Priscilla Furth / Margaret Jones)

- **Genomic and Non-Genomic Signaling by Nuclear Hormone Receptors**
(*Rakesh Kumar* / Jan-Åke Gustafsson / Ellis Levin)
- **Genomics, Proteomics, and Metabolomics**
(*Shuk-Mei Ho*)
- **Hormone Metabolism**
(*Shiuan Chen* / *Rajeshwar Tekmal* / Jose Russo / Thomas Sutter / Bob Brueggemeier)
- **Keynote Speaker**
(*Donald Coffey* / Gerald Cunha)

HUMAN GENETICS & GENOMICS
SALVE REGINA UNIVERSITY
NEWPORT, RI
JUL 24-29, 2005
ERIC GREEN, CHAIR
JAMES LUPSKI, VICE CHAIR

- **Opening Keynote**
(Francis Collins)
- **Human Haplotype Structure**
(David Altshuler / Mark Daly / Yusuke Nakamura / David Cox / Peter Donnelly / Matt Hurles)
- **Monogenic Disorders**
(Bob Nussbaum / Steve Warren / Raju Kucherlapati / Stuart Orkin)
- **Oligogenic Disorders**
(Nicholas Katsanis / Aravinda Chakravarti / Eric Boerwinkle)
- **Therapeutics for Genetic Disorders**
(Michael Sereda / Jean Bennet)
- **Human Genomics**
(Tom Gingeras / Eric Green / Eddy Rubin / Evan Eichler / David Page)
- **Genome Dynamics**
(Nigel Carter / John Moran / Mike Wigler / Jim Sikela / Jim Lupski)
- **Chromosome Biology**
(Hunt Willard / Wendy Bickmore / Molly Przeworski)
- **Closing Keynote**
(Mario Capecchi)

HYDROGEN-METAL SYSTEMS
COLBY COLLEGE
WATERVILLE, ME
JUL 10-15, 2005
RONALD GRIESSEN &
TERRENCE UDOVIC, CO-CHAIRS
CRAIG JENSEN &
KLAUS YVON, CO-VICE CHAIRS

- **Destabilized Hydrides/Imides**
(*Robert Bowman* / John Vajo / Shin-ichi Orimo)
- **Complex Hydrides**
(*Volodymyr Yartys* / Wojciech Grochala / *Ragaiy Zidan* / Andreas Züttel / Hendrik Brinks)
- **New Hydrogen-Storage Materials**
(*Keith Ross* / Wendy Mao)
- **First-Principles Studies**
(*Mei-Yin Chou* / Taner Yildirim / *Michèle Gupta* / Miquel Salmeron / Manos Mavrikakis)
- **Hydrides for Batteries**
(*Annick Percheron-Guégan* / Peter Notten / Michel Latroche)
- **Thin Films**
(*Astrid Pundt* / Cyril Chacon / *Bjorgvin Hjörvarsson* / Bernard Dam)
- **Hydrogen Mobility**
(*Alexander Skripov* / Klaus-Dieter Kreuer / Gunter Majer)
- **Magnetism/Novel Measurement Techniques**
(*Peter Vajda* / Igor Goncharenko / *Jack Rush* / Zamir Gavra / Gerhard Krexner)
- **Hydrogen-Storage: Global Views**
(*Yuh Fukai* / Nobuhiro Kuriyama / *Gary Sandrock* / Scott Jorgensen)

IMMUNOCHEMISTRY & IMMUNOBIOLOGY
THE QUEEN'S COLLEGE
OXFORD, UK
AUG 7-12, 2005
GARY KORETZKY, CHAIR
FIONA POWRIE, VICE CHAIR

- **B and T Cell Development**
(*Meinrad Busslinger* / Katia Georgopoulos / Rudolf Grosschedl / Juan Carlos Zuniga-Pflucker)
- **Immune Cell Recognition**
(*Mitchell Kronenberg* / D. Branch Moody / Ian Wilson / Max Cooper)
- **Innate Immune Responses**
(*Lewis Lanier* / Bruce Beutler / Christine Biron / Ruslan Medzhitov)
- **Immune Cell Activation**
(*Doreen Cantrell* / Andre Veillette / Jurge Tschopp / Warren Leonard)
- **Memory and Effector Function**
(*Steven Reiner* / Rafi Ahmed / Richard Flavell / Susan Swain)
- **Cell Positioning in the Immune System**
(*Jason Cyster* / Takashi Nagasawa / Thomas Boehm / Dan Littman)
- **In Vivo Imaging of Immune Responses**
(*Ronald Germain* / Matthew Krummel / Sebastian Amigorena / Ellen Robey)
- **Host/Pathogen Interactions**
(*Anne O'Garra* / Jorge Galan / Caetano Reis e Sousa / Emil Unanue)
- **Termination of Immune Responses**
(*Fiona Powrie* / Alexander Rudensky / Arlene Sharpe / Michael Lenardo)

INHIBITION IN THE CNS
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUL 3-8, 2005
ISTVAN MODY, CHAIR
CHRIS MCBAIN, VICE CHAIR

- **Plenary Lecture I**
(Peter Somogyi)
- **Anatomy and Physiology of Interneurons I**
(*Tamás Freund* / John Rubenstein / Rosa Cossart / Zoltán Nusser / Alain Marty)
- **Structure and Function of GABA Receptors I**
(*Robert Macdonald* / Cynthia Czajkowski / Richard Olsen)
- **Data Blitz**
(*Chris McBain*)
- **Structure and Function of GABA Receptors II**
(*Ruth McKernan* / Neil Harrison / Stephen Moss / David Weiss / Werner Sieghart)
- **Inhibitory Synaptic Transmission I**
(*Alex Thomson* / Massimo Scanziani / Matt Jones / Peter Jonas / Mark Farrant)
- **Inhibitory Synaptic Transmission II**
(*Ivan Soltesz* / Mu-Ming Poo / Bryndis Birnir / Dimitri Kullman / Jean-Claude Lacaille / Kai Kaila)
- **Inhibition, Networks and Plasticity**
(*György Buzsáki* / Richard Miles / Robert Pearce / Kevin Staley / Yu-Tian Wang)
- **Pathology of Inhibition**
(*Stefano Vicini* / Sheryl Smith / Douglas Coulter / Steven Petrou / Hanns Möhler / Carolyn Houser / Yves DeKoninck)
- **Plenary Lecture II**
(Hannah Monyer)

INORGANIC CHEMISTRY
SALVE REGINA UNIVERSITY
NEWPORT, RI
JUL 17-22, 2005
GEORGE STANLEY, CHAIR
BAHRAM MOASSER, VICE CHAIR

- **Session 1**
(*Bruce Bursten* / Jaqueline Kiplinger / David Vicic / Paul J. Chirik)
- **Session 2**
(*Tracy Hanna* / Julia Chan / Janis Louie / Jonathan Wilker / Dan Mindiola)
- **Session 3**
(*Jennifer Petoff* / Monique Krom / Mark Mason / Jeffrey Long)
- **Session 4**
(*George Stanley* / Roy Periana / Cassandra Fraser / Pascal Le Floch)
- **Session 5**
(William Buhro / Michael D. Fryzuk / Christopher Cummins)
- **Session 6**
(*Andrew Maverick* / William Geiger / Makoto Fujita / Peter Caravan)
- **Session 7**
(*Kim Dunbar* / Karl Wieghardt / Susan Kauzlarich / Stephen Koch)
- **Session 8**
(*Jim Mayer* / Russell Hughes / Francis DiSalvo / David Tyler / Clifford Kubiak)
- **Session 9**
(*Bahram Moasser* / Richard Eisenberg / Harry Gray)

INORGANIC GEOCHEMISTRY
PROCTOR ACADEMY
ANDOVER, NH
JUL 31-AUG 5, 2005
JEAN CLINE & STEVE GARWIN, CO-CHAIRS
CHRISTOPH HEINRICH, VICE CHAIR

- **Economic Geology: The View from Industry**
(*John Thompson* / Greg Hall / Jon Hronsky / Doug Kirwin)
- **Global Tectonics, Magmatism and the Generation of Magmatic-Hydrothermal Fluids**
(*David John* / Cornel de Ronde / Robert Loucks / Yasushi Watanabe)
- **Global Tectonic Settings of Ore-Fluid Generation in Oceanic and Continental Basins**
(*Murray Hitzman* / Ross Large / Jamie Wilkinson)

- **Chemical Processes Controlling Fluid and Solute Transport**
(*Phil Candela* / Frank P. Bierlein / Daniel Core / John Mavrogenes)
- **Physical Factors Driving and Localizing Fluid Flow**
(*Larry Cathles* / Larryn Diamond / Thomas Driesner & Sebastian Geiger)
- **Chemical Traps for Economic Metal Precipitation**
(*Mark Reed* / Robert Moritz & K. Kouzmanov / Brian Rusk, Mark Reed & John Dilles / John Muntean & Jean Cline)
- **Physics of Fluid Focusing and Hydrothermal Heat Balance**
(*Jeremy Richards* / Stephen Cox / Raul Madrid)
- **Remobilization and Secondary Enrichment of Metals in the Supergene Environment**
(*James Saunders* / George Brimhall / Steve Enders / Ming-Kuo Lee)
- **Research Frontiers in Hydrothermal Processes and Economic Geology**
(*Antonio Arribas* / Nick Archibald / Robert Bodnar / Steve Kesler / Richard Sillitoe / Moira Smith)

INTERIOR OF THE EARTH
MOUNT HOLYOKE COLLEGE
SOUTH HADLEY, MA
JUN 12-17, 2005
LARS STIXRUDE, CHAIR
GORAN EKSTROM, VICE CHAIR

- **Physics and Chemistry of the Core-Mantle Boundary**
(*Thorne Lay* / Don Helmberger / Kei Hirose)
- **Structure and Evolution of the Core**
(*Bruce Buffett* / Dion Heinz / Miaki Ishii)
- **Geomagnetic Field and the Geodynamo**
(*Cathy Constable* / Andrew Jackson / Johannes Wicht)
- **Temperature and Composition of the Lower Mantle**
(*Guy Masters* / Guillaume Fiquet / Guust Nolet / Paul Tackley)
- **The Transition Zone**
(*Barbara Romanowicz* / Daniel Frost / Jeroen Ritsema)
- **Plate Tectonics and Deep Mantle Structure**
(*Michael Manga* / John Lassiter / Adrian Lenardic / Shijie Zhong)
- **Planetary Interiors**
(*Catherine Johnson* / Marc Parmentier / Sabine Stanley)
- **Evolution of the Mantle**
(*Erik Hauri* / Paul Asimow / Al Hofmann / Don Porcelli)
- **Unresolved Problems**
(*Alex Halliday* / Bernard Wood / David Stevenson)

LASER DIAGNOSTICS IN COMBUSTION
MOUNT HOLYOKE COLLEGE
SOUTH HADLEY, MA
JUL 31-AUG 5, 2005
MARK ALLEN, CHAIR
PAUL EWART, VICE CHAIR

- **Advances in Imaging Techniques**
(*Campbell Carter* / Jonathan Frank / Alaa Omrane)
- **New Sources and Applications**
(*Tom Settersten* / Nils Hansen / Robert Walker / Dahv Kliner)
- **Turbulence and Diagnostics Interactions**
(*Andreas Dreizler* / Dirk Geyer / Noel Clemens)
- **Laser Diagnostics of Biological Phenomena**
(*Jurgen Wolfrum* / Clemens Kaminski / Andreas Brockhinke / Houston Miller)
- **Particulates**
(*Gregory Smallwood* / Joe Mauderly / Kim Prather)
- **Engine Applications**
(*Volker Sick* / Jay Jeffries / Wieland Koban / Scott Sanders)
- **Diagnostics in Multi-Phase Flows**
(*Christof Schulz* / Hope Michelsen / Michael Drake)
- **Non-Linear Optics**
(*Michael Brown* / Bob Lucht / Zhungshan Li / Robert Stevens)
- **Hot Topics: A Selection From the Contributed Posters**
(*Paul Ewart*)

LIPIDS, MOLECULAR & CELLULAR BIOLOGY OF
KIMBALL UNION ACADEMY
MERIDEN, NH
JUL 24-29, 2005
JEAN VANCE, CHAIR
CHARLES MARTIN, VICE CHAIR

- **Mechanisms of Lipid Homeostasis**
(*William Dowhan* / *Dennis Vance* / Joseph Goldstein / Michael Brown)
- **Cholesterol Metabolism**
(*Laura Liscum* / David Russell / Jerome Strauss / Randy Hampton / Russell DeBose-Boyd)
- **Interorganelle Lipid Transport**
(*Dennis Voelker* / Masahiro Nishijima / Richard Pagano / Gerrit van Meer)
- **Phospholipid Metabolism**
(*Suzanne Jackowski* / Howard Goldlfine / Rosemary Cornell / Neale Ridgway / Arnold Strauss)
- **Derivatives of Fatty Acids**
(*Edward Dennis* / Thomas McIntyre / William Smith)
- **Fatty Acid Biosynthesis**
(*Chuck Rock* / John Cronan / Diego de Mendoza / David Bernlohr / James Ntambi)
- **ABC Transporters and Transmembrane Lipid Flux**
(*Chris Raetz* / Peter Edwards / Chip Davis / Helen Hobbs)
- **Triacylglycerol Metabolism, Obesity and Diabetes**
(*Rosalind Coleman* / Dawn Brasaemle / Karen Reue / Hei-Sook Sul / Chris Newgard)
- **Regulation of Lipid Metabolism**
(*George Carman* / Bruce Spiegelman / Carl Sparrow / Charles Martin)

LIQUID CRYSTALS
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUN 19-24, 2005
OLEG LAVRENTOVICH, CHAIR
GREGORY CRAWFORD, VICE CHAIR

- **Biological Aspects**
(Françoise Livolant / Cyrus Safinya / Gerard Wong)
- **Thin Films and Membranes**
(Sarah Keller / Ka Yee Lee / Yuka Tabe)
- **Sensors and Drugs Delivery**
(Nicholas Abbott / Joseph Zasadzinski / Jonathan Selinger)
- **Self-Assembly and New Phases**
(Noel Clark / Gregory Grason / Carsten Tschierske)
- **Defects and Patterns**
(Maurice Kleman / Robert Kusner)
- **Theories and Simulations of Molecular Order**
(Lech Longa / Claudio Zannoni)
- **Colloids and Liquid Crystals**
(Igor Musevich / Seth Fraden)
- **Liquid Crystalline Elastomeres**
(Patrick Mather / Robert Meyer)
- **Optics and Electrooptics**
(Diederik Wiersma / Dirk Broer)

LIQUIDS, CHEMISTRY & PHYSICS OF
HOLDERNESS SCHOOL
PLYMOUTH, NH
JUL 24-29, 2005
GRAHAM FLEMING, CHAIR
DAVID REICHMAN, VICE CHAIR

- **Complex Fluids**
(David Grier / Mike Cates)
- **Quantum Processes in Liquids**
(Eran Rabani / Brigitte Whaley / Nancy Makri)
- **Ionic Liquids**
(Paul Madden / C. Austen Angell)
- **Glass Transition**
(Nathan Israeloff / Juan P. Garrahan / Ranko Richert)
- **Confined Liquids**
(Steve Granick / Rustem Ismagilov)
- **Liquids/Biological Interfaces & Systems**
(L. Mahadevan / Ka Yee C. Lee / Gerard Wong)
- **Equilibrium Properties from Non-Equilibrium Experiments**
(Gerhard Hummer / Jan Liphardt)
- **Nonlinear Spectroscopic Studies of Liquid Dynamics**
(Robin Hochstrasser / Phillip Geissler / Martin Zanni)
- **Final Presentation**
(Robert Zwanzig)

MAGNETIC RESONANCE
CONNECTICUT COLLEGE
NEW LONDON, CT
JUN 5-10, 2005
MICHAEL MEHRING, CHAIR
SHIMON VEGA, VICE CHAIR

- **From Physics to Medicine**
(*Michael Mehring* / Erwin Hahn / Kamil Ugurbil)
- **Structural Investigations with Pulsed NMR and EPR**
(*Kamil Ugurbil* / Jack Freed / Beat Meier / Tatyana Polenova)

- **External and Remote Detection**
  (*Alex Pines* / Bernhard Blümich / Song I-Han / Yung-Ya Lin)
- **From Strongly Correlated Solids to Proteins**
  (*Robert G. Griffin* / Claude Berthier / Jürgen Haase / Kurt Zilm / David Weliky)
- **High Frequency and Complex Pulse Sequences for Structure Determination in Biological Systems**
  (*Beat Meier* / Edgar Groenen / Judith Herzfeld / Steffen J. Glaser)
- **Single Electron Spin Detection and Spin Manipulations in Semiconductors**
  (*Claude Berthier* / Dan Rugar / H.W. Jiang / Martino Poggio)
- **Tales and Novel Concepts of NMR**
  (*Warren Warren* / Malcolm Levitt / Jonathan Jones / Lucio Frydman)
- **From Microtesla NMR to EPR/NMR Structure-Function Investigations**
  (*Kurt Zilm* / John Clarke / Robert Tycko / Tim Cross / Yuri D. Tsetkov)
- **BOOMERANG and Structural Investigations**
  (*Shimon Vega* / Daniel Weitekamp / Thomas Prisner / Lyndon Emsley)

MALARIA
THE QUEEN'S COLLEGE
OXFORD, UK
AUG 21-26, 2005
ANDY WATERS, CHAIR
KEVIN MARSH, VICE CHAIR

- **Molecular Aspects of Drug Resistance and Drug Targets**
  (*Nick White* / Steve Ward / Michael Ferdig / John Hyde)
- **Genomes and Post Genome Analyses**
  (*Dan Carucci* / Chris Newbold / Jane Carlton / Xinzhuan Su / Ray Hui)
- **Molecular Aspects of Infection**
  (*David Conway* / Stefan Kappe / Ute Frevert / Dominique Soldati)
- **Invasion**
  (*Tony Holder* / Alan Cowman / Chetan Chitnis / Masao Yuda / Maria Mota)
- **Transmission from Host to Vector**
  (*Geoff Targett* / Oliver Billker / Bob Sinden / Ken Vernick)
- **Immunity and Malaria**
  (*Eleanor Riley* / Jean Langhorne / Brendan Crabb / Laurent Renia / Chris Hunter)
- **Gene Expression**
  (*Dyann Wirth* / Kirk Deitsch / Artur Scherf / Henk Stunnenberg)
- **Molecular Aspects of Pathology**
  (*Kevin Marsh* / Alex Rowe / Pete Bull / Joe Smith / Kasturi Haldar)
- **Parasite Virulence**
  (*Brian Greenwood* / Richard Carter / Andrew Read / David Sibley)

MAMMARY GLAND BIOLOGY
SALVE REGINA UNIVERSITY
NEWPORT, RI
JUN 12-17, 2005
JEFFREY POLLARD, CHAIR
ROBERT CLARKE, VICE CHAIR

- **Keynote Speaker**
  (*Jeffrey W. Pollard* / Tak Mak)
- **Epithelial Mesenchymal Interactions in Breast Cancer**
  (*Terri Wood* / Lewis Chodosh / Claire Lewis / John Condeelis / Derek Radisky)
- **Developmental Transitions I**
  (*Charles Streuli* / Lothar Hennighausen / Anthony Brown / Caroline Alexander)
- **Developmental Transitions II**
  (*Pam Cowen* / Robert Rhoads / Christine Watson / Cynthia Zahnow / Gertraud Robinson)
- **Sex Steroid Hormone Regulation in Mammary Development and Cancer**
  (*Robert Clarke* / Sandy Haslam / Leena Hilakivi-Clarke / Gabriela Dontu)
- **Lactation**
  (*Margaret Neville* / John Wysolmersky / Jim McManaman / Sarah Berry / Christopher Linington)
- **Peptide Hormone Signaling and Growth Factors**
  (*Paul Kelley* / Christopher Ormandy / Nelson Horseman / Mary Helen Barcellos-Hoff / Sacha Howell)
- **Cell Cycle, DNA Repair Pathways, Chromosomal Abnormalities and Breast Cancer**
  (*Jeffrey Rosen* / Joe Jerry / Joe Grey / Ralph Scully / Elizabeth Musgrove)
- **Keynote Speaker**
  (*Jeffrey W. Pollard* / Robert Weinberg)

MATRIX METALLOPROTEINASES
BIG SKY RESORT
BIG SKY, MT
AUG 28-SEP 2, 2005
ZENA WERB, CHAIR
CARLOS LOPEZ-OTIN, VICE CHAIR

- **The Metalloproteinase Toolbox: Structural Biology of Enzymes and Substrates, the Degradome, Visualizing MMPs**
  (*Zena Werb* / Ben Cravatt / Chris Overall / Ambra Pozzi / Lynn Matrisian)
- **Metalloproteinases in Stem Cells, Development and Aging**
  (*Lynn Matrisian* / Judy Campisi / Andrea Page-McCaw / Kiyoji Nishiwaki / Beate Heissig / Joe Madri / Judith Kimble)
- **Metalloproteinases in Inflammation**
  (*Dylan Edwards* / Ian Clark / Farrah Kheradmand / Rama Khokha)
- **Metalloproteinases in the Nervous System-Neurodegenerative Diseases, Memory and Aging**
  (*Chris Overall* / Leszek Kaczmarek / Gary Rosenberg / Kathy Conant / Falk Fahrenholz / Voon Wee Yong)
- **ADAMS and ADAMTS Families in Development and Disease**
  (Ulla Wewer / Marcos Milla / Carl Blobel)
- **Metalloproteinases in Cancer**
  (Mikala Egeblad / Agnes Noel / Lisa Coussens)
- **Inhibitors of Metalloproteinases-TIMPs and Others**
  (*Rama Khokha* / Vincent Dive / Richard Dyer / Caroline Owen)
- **Metalloproteinase Gene Regulation, Cell Biology Activation and Receptors**
  (*Rafi Fridman* / Alex Ullrich / Nabil Seidah / Carlos Lopez-Otin / Alicia Arroyo / Catherine Muller)
- **MMPs- The Next Generation**
  (*Carlos Lopez-Otin* / Roger Y. Tsien / Chris Overall / Dylan Edwards)

MECHANISMS OF CELL SIGNALLING
HONG KONG UNIVERSITY OF SCIENCE & TECHNOLOGY
HONG KONG, CHINA
JUN 12-17, 2005
SHUH NARUMIYA, CHAIR
JONATHAN CHERNOFF, VICE CHAIR

- **Signal Transduction and Cytoskeletal Dynamics**
  (*David Pellman* / Mike Rosen / Fred Wittinghofer / Tadaomi Takenawa)
- **Cell Polarity and Migration I**
  (*Alan Hall* / Rick Firtel / Alexander van Oudenaarde / Klaus Hahn / Zhenbiao Yang)
- **Cell Polarity and Migration II**
  (*Greg Gundersen* / Fred Chang / Tetsu Akiyama / Atsuko Kodama)
- **Cell Adhesion**
  (*Yoshimi Takai* / Hans Bos / Martin Schwartz / Valerie Weaver)
- **Cell Proliferation and Malignant Transformation I**
  (*Dafna Bar-Sagi* / Richard Marais / David Tuveson / Fuyuhiko Tamanoi)
- **Cell Proliferation and Malignant Transformation II**
  (*Chris Marshall* / Yukihiko Kabuyama / John Blenis / Jonathan Chernoff)
- **Axon Guidance and Synaptic Plasticity**
  (*Linda van Aelst* / Yukiko Goda / Mu-Ming Poo / Yuh-Nung Jan / Didier Job)
- **Mitosis and Cytokinesis**
  (*Tony Hyman* / Bruno Goud / Ed Manser / Robert Rottapel)
- **GTPases in the Immune System**
  (*Doreen Cantrell* / Nagahiro Minato / Victor Tybulewicz)

MECHANISMS OF MEMBRANE TRANSPORT
TILTON SCHOOL
TILTON, NH
JUN 5-10, 2005
ROBERT STROUD, CHAIR
PETER MALONEY, VICE CHAIR

- **Principles of Membrane Protein Construction**
  (*Michael Wiener* / Don Engelman / Steven White / James Bowie)
- **The Amt/MEP/Rh family and Gas Transport**
  (*Robert Stroud* / Shahram Khademi / Connie Westhoff / Walter Boron)
- **Translocation of Proteins**
  (Michael Wiener / Thomas Rapaport)
- **Channels that are Transporters and Vice Versa**
  (Chris Miller / Tzyh-Chang Hwang / David Gadsby / Michael Kavanaugh / David Clapham)
- **ABC Transporters**
  (*Phil Thomas* / Amy Davidson / Shimon Schuldiner / Kaspar Locher)
- **Major Facilitators**
  (*Peter Maloney* / Manuel Palacin / Ron Kaback / Susan Buchanan)
- **Regulation of Transporters**
  (*Robert Edwards* / Barouk Kanner / Cecilia Canessa / David Gadsby / Bernd Fakler / Nancy Andrews)
- **Synaptic Transporters**
  (Sandy Bajjalieh / Jean-Yves Lapointe / Robert Edwards / Eric Gouaux / Susan Amara)
- **P-Type ATPases**
  (*David Stokes* / Rhoda Blostein / Barry Rosen / Jose Arguello / Svetlana Lutsenko)

- **Mechanisms of Water Transport**
  (Henning Stahlberg / Thomas Walz / William Harries)
- **Late Breaking News**

MECHANOTRANSDUCTION & GRAVITY SIGNALING IN BIOLOGICAL SYSTEMS
THE UNIVERSITY OF NEW ENGLAND
BIDDEFORD, ME
JUL 24-29, 2005
MARTIN CHALFIE, CHAIR
PAUL BLOUNT &
GLORIA MUDAY, CO-VICE CHAIRS

- **Mechanosensing in Animals, Plants and Microbes**
  (Ching Kung / Maurice Kernan / Ruth Anne Eatock)
- **Cellular and Tissue Response to Mechanical Stimuli or Gravity**
  (Simon Gilroy / Jeffrey Dazen / Mark Johnson)
- **Mechanosensitive Channels I**
  (Eduardo Perozo / Ian Booth)
- **Genetic Approaches to Gravity Signaling**
  (Elizabeth Haswell / Jiri Friml)
- **Mechanical and Gravity Signal Transduction**
  (Natasha Raikhel / Angus Murphy / Mike Gustin)
- **Mechanosensitive Channels II**
  (Miriam Goodman / David Corey / Klaus Schulten)
- **Genetic Approaches to Mechanotransduction**
  (Teresa Nicolson / Cori Bargmann)
- **Hot Topics in Mechanotransduction and Gravity Signaling**

MEDICINAL CHEMISTRY
COLBY-SAWYER COLLEGE
NEW LONDON, NH
AUG 7-12, 2005
S. DAVID KIMBALL, CHAIR
JOHN LOWE, VICE CHAIR
GEORGE HARTMAN, VICE CHAIR ELECT

- **Kinase Inhibitors**
  (*Andrew Mortlock* / Walter Ward / Paul Manley / Michael Kaufman / Brian Werneberg)
- **The Hit-to-Lead Process**
  (*Amy Ripka* / Samuel Gerritz / Brian Shoichet / Bill Michne / Dennis Underwood)
- **Biomarkers and PET Imaging**
  (*Balu Balusubramanian* / Chet Mathis / Doug Dischino / Richard Hargreaves)
- **HCV Antivirals**
  (*Michael Bos* / Ralf Bartenschlager / Robert Perni / Pierre Beaulieu / Dick Storer)
- **New Directions in Antibiotics**
  (*John Primeau* / Paul Aristoff / Dean Stamos / Zhengyu Yuan)
- **Protease Inhibitors for Treating Pulmonary Inflammatory Disorders**
  (*Bruce Maryanoff* / Clive Page / Michael Greco / Zhongli Gao / John Link)
- **New Treatments for Ophthalmological Disorders**
  (*Peter Klimko* / Jun Inoue / Yongxin Han / Jesse May)
- **Special Topics**
  (*Mary Mader*)
- **Chair's Talk**
  (*Roy Vagelos*)

MICROBIAL POPULATION BIOLOGY
PROCTOR ACADEMY
ANDOVER, NH
JUL 17-22, 2005
MARGARET RILEY, CHAIR
PAUL RAINEY, VICE CHAIR

- **How Far Have We Come; The Past Twenty Years of Microbial Population Biology**
  (Bruce Levin / Dan Hartl / Dan Dykhuizen / John Roth / Monica Riley)
- **Phage Diversity and Evolution**
  (Jim Bull / Roger Hendrix / Holly Wichman / Susanna Twiddy)
- **Protist Diversity and Evolution**
  (Graham Bell / Lynn Margulis / Laura Katz)
- **Microbial Ecology**
  (Colleen Cavanaugh / Sallie Chisolm / Peter Morin / Takema Fukatsu)
- **Cooperativity Among Microbes**
  (Greg Velicer / Ben Kerr / Ashleigh Griffin)
- **Ecology and Evolution of Infectious Disease**
  (Abigail Saylers / Nicole Perna / Julian Parkhill / Carl Bergstrom)
- **Genome Enabled Studies**
  (Jeffrey Blanchard / Fred Blattner / Jonathon Eisen / Bernhard Palsson)
- **Phenotype/Genotype Mapping**
  (Dom Schneider / Rob Dorit / Rich Lenski)
- **Evolution of Sex**
  (Rosemary Redfield / Lin Chao / Sally Otto)

MICROFLUIDICS, PHYSICS & CHEMISTRY OF
MAGDALEN COLLEGE
OXFORD, UK
AUG 21-26, 2005
LAURIE LOCASCIO, CHAIR
SABETH VERPOORTE, VICE CHAIR

- **Single Molecule Manipulation and Measurement**
  (*Sabeth Verpoorte* / Yoshinobu Baba / Jean-Loius Viovy)
- **Multiphase Systems**
  (*Steven Haswell* / David Weitz / Wyatt Vreeland / Rustem Ismagilov / Kathleen Stebe)
- **Nanobiotechnology**
  (*Luke Lee* / Harold Craighead / Paul Cremer)
- **Pushing the Boundaries of Separation**
  (James Landers / Gert Desmet / Annelise Barron / Anup Singh)
- **Integrated Systems**
  (*Brian Kirby* / Richard Mathies / Mark Burns)
- **Nano and Microscale Transport**
  (*Gary Slater* / Hans Hermann Gerdes / Albert van den Berg / Don DeVoe / Sandra Troian)
- **Crossover**
  (*Paul Yager* / Olga Vinogradova / David Grier)
- **Cells in Microfluidics**
  (*Peter Wilding* / Daniel Chiu / Minoru Seki / Jon Cooper / Sangeeta Bhatia)
- **Nanofluidics versus Microfluidics**
  (*Laurie Locascio* / Andreas Manz / Jan Eijkel)

MOLECULAR & CELLULAR BIOENERGETICS
KIMBALL UNION ACADEMY
MERIDEN, NH
JUN 26-JUL 1, 2005
PATRICIA KANE, CHAIR
STANLEY DUNN, VICE CHAIR

- **Respiratory Chain Complexes: Structure, Function, and Mechanism**
  (*Shelagh Ferguson-Miller* / Leo Sazanov / Ulrich Brandt / Edward Berry)
- **Bioenergetics and Disease**
  (*David Nicholls* / Eric Schon / Martin Brand / Daniel Ricquier / Valina Dawson)
- **Metal Ion Homeostasis and Mitochondrial Function**
  (Jerry Kaplan / Sabeeha Merchant)
- **V- and F-type ATPases: Structure, Function, and Mechanism**
  (*Brian Cain* / Stephan Wilkens / Bettina Bottcher / Nathan Nelson / Masasuke Yoshida)
- **Sensing and Regulating pH Gradients In Vivo**
  (*Michael Forgac* / Rajini Rao / Fiona Karet / Raul Martinez-Zaguilan)
- **Poster Discussion Workshops**
  (*L. Shannon Holliday* / *Terry Krulwich* / *Diego Gonzalez-Halphen* / *Robert Gennis*)
- **Biosynthesis and Assembly of Respiratory Chain Complexes**
  (*Rosemary Stuart* / Antoni Barrientos / Carlos Moraes / Thomas Langer)
- **Genomic and Proteomic Approaches to Bioenergetics**
  (*Andre Goffeau* / Douglas Wallace / Volker Muller / Brad Gibson / Brigitte Meunier)
- **New Frontiers in Mitochondria Biology**
  (*Peter Pedersen* / Ronald Butow / David Chan / Vamsi Mootha)

MOLECULAR MECHANISMS OF MICROBIAL ADHESION
SALVE REGINA UNIVERSITY
NEWPORT, RI
AUG 7-12, 2005
PASCALE COSSART &
ROBERTO KOLTER, CO-CHAIRS
BONNIE BASSLER &
ARTURO ZYCHLINSKY, CO-VICE CHAIRS

- **Opening Lectures**
  (Jules Hoffmann / Jeff Gordon)
- **Structure/Localization**
  (*Staffan Normark* / Maria Sandkvist / Christine Jacobs / Eduardo Groisman / Guy Cornelis)
- **Microbe-Plant Interactions**
  (*Staffan Normark* / Brian Staskawicz / Sharon Long / Jeff Dangl)
- **Multicellularity and Biofilms**
  (*Philippe Sansonetti* / Ute Römling / Iñigo Lasa / George O'Toole / Jean-Marc Ghigo)
- **Immunity**
  (*Philippe Sansonetti* / Arturo Zychlinsky / Stephen Girardin / Michael Starnbach)
- **Eukarya-Bacteria Signaling**
  (*Brett Finlay* / Dan Portnoy / Deborah Hogan / Norma Andrews / Javier Pizarro-Cerda)
- **Toxins and Virulence Factors**
  (*Brett Finlay* / Gisou van der Goot / Emmanuel Lemichez / Michael Caparon)
- **Intra- and Extra- Cellular Signaling**
  (*Jorge Galán* / Richard Losick / Bonnie Bassler / Gisela Storz / Diego de Mendoza)

- **Pneumococcus-Streptococcus**
(*Jorge Galán* / Birgitta Henriques / Paul Sullam)

MOLECULAR MEMBRANE BIOLOGY
PROCTOR ACADEMY
ANDOVER, NH
JUL 10-15, 2005
SANDRA SCHMID, CHAIR
BENJAMIN GLICK, VICE CHAIR

- **Membrane Fusion**
(*Bill Wickner* / Ed Chapman / Reinhard Jahn / Margaret Kielian)
- **Diverse Translocation Machineries**
(*Tom Rapoport*/ Hidde Ploegh / Nikolaus Pfanner / Rick Rachubinski / Art Johnson)
- **Intracellular Sorting Mechanisms**
(*Peter Walter* / Shou-ou Shan / Hugh Pelham / Linda Hicke)
- **Organizing Membrane Subdomains**
(*Ira Mellman* / Rob Parton / Matthias Weiss / Catherine Rabouille)
- **Membrane Bending/Fission**
(*Janet Shaw* / Alberto Luini / Harvey McMahon / Michael Kozlov)
- **Conformational Dynamics at the Membrane**
(*Bill Balch* / Don Engelman / David W. Andrews / Ehud Isacoff / Diane Papazian)
- **Lipids and Compartment Identity**
(*Pietro DeCamilli* / Jean Gruenberg / Temo Kurzchalia / Scott Emr)
- **Organelle Biogenesis and Dynamics**
(*Graham Warren* / Jodi Nunnari / Jennifer Lippincott-Schwartz / Sean Munro / Judith Klumperman)
- **Transport Carrier Formation**
(*Randy Schekman* / Bruno Antonny / Juan Bonifacino / Vivek Malhotra)

MOLECULAR THERAPEUTICS OF CANCER
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUL 17-22, 2005
YVES POMMIER, CHAIR
ALAN EASTMAN, VICE CHAIR

- **Targeting the Genome**
(*Christian Bailly* / Christian Bailly / Laurence Hurley / John Hartley)
- **Translation Initiation Pathways for Cancer Therapy**
(*Peter Houghton* / Peter Houghton / Chris Proud / Sara Kozma / Jerry Pelletier)
- **c-myc and Transcription**
(*Peter K. Vogt* / Peter K. Vogt / Edward Prochownik / Lubomir Vassilev / Kevin Gardner)
- **Tantalizing Thanatos-Cell Death as a Therapeutic Goal**
(*Guido Kroemer* / Guido Kroemer / John C. Reed / Douglas R. Green / Craig Thompson)
- **Survival Pathways**
(*Phillip Dennis* / Philip Dennis / William Pao / Shigeki Miyamoto)
- **The p53-Mdm2 and Stress Response Pathways**
(*Wafik S. El-Deiry* / Thanos Halazonetis / Andrei Gudkov / Alan Weissman / Wafik El-Deiry)
- **Exploiting Hypoxia in Cancer Treatment**
(*J. Martin Brown* / Adrian Harris / Giovanni Mellilo / William Wilson)
- **Hip-Hop on Microtubules: Cytoskeleton Takes Center Stage in Cancer Therapy**
(*Paraskevi (Evi) Giannakakou* / Ken Downing / Paraskevi Giannakakou / Tito Fojo / Gregg Gundersen)
- **Unconventional Therapeutic Chemical Approaches**
(*Greg Verdine* / Greg Verdine / Julian Adam / Saul Rosenberg)

MOLYBDENUM & TUNGSTEN ENZYMES
THE QUEEN'S COLLEGE
OXFORD, UK
JUL 10-15, 2005
MICHAEL JOHNSON &
TRACY PALMER, CO-CHAIRS
CAROLINE KISKER &
ALASTAIR MCEWAN, CO-VICE CHAIRS

- **Keynote Speakers**
(K.V. Rajagopalan / Les Dutton)
- **Molybdenum Cofactor Biosynthesis**
(*Ralf Mendel* / Guenter Schwarz / Petra Haenzelmann / Herman Schindelin / Silke Leimkuhler / Emilio Fernandez)
- **Sulfite Oxidases**
(*Caroline Kisker* / John Enemark / Sue Bailey / Partha Basu)
- **DMSO Reductase Family**
(*Al McEwan* / Fraser Armstrong / Chantal Iobbi-Nivol / Dick Holm)
- **Nitrate Reductases**
(*David Richardson* / Joel Weiner / Katrin Fischer / Simon De Vries / Jose Moura)
- **Xanthine Oxidase Family**
(*Russ Hille* / Takeshi Nishino / Florian Bittner / Matthias Boll / Marty Kirk / Charlie Young)
- **Tungsten Enzymes**
(*Dave Garner* / Mike Adams / Peter Kroneck / Maria Romao)
- **Applied, Environmental and Medical Aspects**
(*Ed Stiefel* / Enrico Garattini / Joanne Santini / Susanne Fetzner / Dave Kelly)

MOTILE & CONTRACTILE SYSTEMS
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUL 10-15, 2005
VLADIMIR GELFAND, CHAIR
MARGARET TITUS, VICE CHAIR

- **Keynote Speaker**
(Joel Rosenbaum)
- **Force Production**
(*Dan Kiehart* / Tom Pollard / Tom Roberts / Michael Sheetz)
- **Centrosome**
(*Tim Stearns* / Alexey Khodjakov / Steve Doxsey / David Agard)
- **Cytoskeletal Diseases**
(*Erika Holzbaur* / Tom Friedman / Frédéric Saudou / Kevin Campbell)
- **Cell Polarity**
(*Carole Parent* / Rong Li / Ian Macara)
- **Cell Division**
(*Claire Walczak* / Kerry Bloom / Don Cleveland / Tarun Kapoor)
- **Intracellular Movements**
(*Lois Weisman* / John Kendrick-Jones / Jon Scholey / Robert Singer / David Drubin / John Hammer)
- **Unusual Cytoskeletons and Host-Pathogen Interactions**
(*Pascale Cossart* / David Sibley / Isabelle Tardieux / Matt Welch)
- **Cytoskeleton and Cell Signaling**
(*Gregg Gundersen* / Shuh Narumiya / Klaus Hahn / Pierre Gönczy)

MULTI-DRUG EFFLUX SYSTEMS
MAGDALEN COLLEGE
OXFORD, UK
AUG 28-SEP 2, 2005
ATTILIO DI PIETRO &
LAURA PIDDOCK, CO-CHAIRS
RICHARD BRENNAN &
SUSAN COLE, CO-VICE CHAIRS

- **Keynote Session - Multi-Drug Resistance Overview: From Bacteria to Man**
(*Laura Piddock* / *Attilio Di Pietro* / Hiroshi Nikaido / Piet Borst)
- **Biological Significance of Multi-Drug Efflux Pumps**
(*Marc Ouellette* / Hendrik Van Veen / Francisco Gamarro / Herman Koepsell)
- **Molecular Basis of Drug Recognition and Transport**
(*Susan Cole* / Melissa Brown / Anne H. Dantzig / Balazs Sarkadi)
- **Structure and Molecular Mechanism of Efflux Pumps**
(*Arnaud Ducruix* / Taiji Nakae / Geoffrey Chang / Anthony M. George)
- **Mechanism of Energy Supply and Coupling to Drug Efflux**
(*Frances Sharom* / Eitan Bibi / Jean-Michel Jault / Christopher Higgins)
- **Regulation of Efflux Pumps**
(*André Goffeau* / Richard Brennan / Scott W. Moye-Rowley / Katleen Scotto)
- **Clinical Relevance**
(*Susan Bates* / *Glenn Kaatz* / Bill Shafer / Antonio T. Fojo / Maria Pia DePasquale)
- **Hot Topics/Controversies: Drug Transport Mechanism**
(*Wil Konings* / *Kim Lewis*)
- **Hot Topics/Controversies: Can Pumps be Blocked? Will it be Efficient on MDR?**
(*David Hooper* / *Olga Lomovskaya*)
- **Reflections on the Past and Glimpses into the Future: Functional Genomics and Pharmacogenomics**
(*Toshihisa Ishikawa* / Estelle Marrer / Rheinhold Kerb / Michael Gottesman)

MUSCLE: CONTRACTILE PROTEINS
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUL 3-8, 2005
LEE SWEENEY, CHAIR
JUSTIN MOLLOY, VICE CHAIR

- **Overview of the Actomyosin Mechanism**
(*James Spudich*)
- **Regulation of Myosin Motors and Muscle Contraction**
(*Kathleen Trybus*)
- **Myosin Movement Beyond the Lever Arm**
(*H. Lee Sweeney*)
- **Myosin Force and Movement Generation**
(*Yale Goldman*)
- **Myosin Force and Movement Generation in Muscle**
(*Malcolm Irving*)

- **Microtubular Motor Function** (*Kazuhiro Oiwa*)
- **Influence of Load on the Actomyosin Cycle of Muscle** (*Michael Ferenczi*)
- **Influence of Load on Processive Motors** (*Claudia Veigel*)
- **Meeting Synthesis** (*Justin Molloy* / *Hugh Huxley*)
- **Poster Review and Discussion** (*Michael Ostap*)

MYCOTOXINS & PHYCOTOXINS
COLBY COLLEGE
WATERVILLE, ME
JUN 19-24, 2005
WAYNE BRYDEN &
STEVE MUSSER, CO-CHAIRS
KATHLEEN REIN &
KENNETH VOSS, CO-VICE CHAIRS

- **Risk Assessment and its Role in Regulatory Decision Making** (*David Miller* / Sarah Henry / Elaine Faustman / Wetzel Gelderblom)
- **Toxicogenomics and Molecular Mechanisms of Toxicity** (*Annie Pfohl-Leszkowicz* / Greg Mayor / Michael Stone / Ed Cleveland / Wolfgang Dekant)
- **Toxin Synthesis and Synthetic Receptors** (*Mark Savard* / Andrea Bordelais / Robert Gawley / Sergey Piletsky)
- **Toxin Induction and Biosynthesis** (*Bill Gerwick* / Nancy Keller / David Sherman / Cynthia Heil / Samuel Lo Chun-Lap)
- **Toxin Mitigation and Control** (*Russell Molyneux* / Kevin O'Shea / Reinhard Fischer / Anne Desjardins / Greg Doucette)
- **Detection Technologies for Organisms and Toxins** (*Ken Voss* / Kathryn Coyne / Kevin James / Rudolf Krska / Chris Taitt)
- **Animal Model Studies I** (*Andrew Gordon* / Janet Benson / Raghu Sharma / Roger Coulombe)
- **Animal Model Studies II** (*Fran Van Dolah* / Jerry Rice / Sven Daenicke / Julie Zaias)
- **Assessing Chronic Exposure to Toxins** (*Robert MacPhail* / Helen Shurz-Rogers)

NATURAL PRODUCTS
TILTON SCHOOL
TILTON, NH
JUL 24-29, 2005
JOSEPH ARMSTRONG, CHAIR
FREDERICK LUZZIO, VICE CHAIR

- **Total Synthesis** (*Dave Askin* / Mark Rizzacasa / Sam Danishefsky)
- **Catalytic Methodology I** (Tim Jamison / Helene Lebel)
- **Catalytic Methodology II** (Dan Yang / Huw Davies / Mikiko Sodeoka)
- **Chemical Ecology/Biology** (*Bradley Moore* / Joern Piel / Nicola Pohl / Jerrold Meinwald)
- **Methodology and Total Synthesis I** (Gary Molander / Corine Aubert)
- **Methodology and Total Synthesis II** (Jim Leighton / Brian Stolz / Erick Carreira)
- **Protein Chemical Biology** (Suzanne Walker / Ben Davis / Herbert Waldmann)
- **Medicinal and Process Chemistry I** (Ann Weber / Karl Hansen)
- **Medicinal and Process Chemistry II** (Milton Brown / Chris Senanayake)
- **Nutraceuticals and Medicinal Plants** (Norman Farnsworth / Qun Yi Zheng / Bruce Lipschutz)
- **Methodology and Total Synthesis III** (Peter Wipf / Eiichi Nakamura)
- **Methodology and Total Synthesis IV** (Andy Evans / Paul Wender)

NEURAL CIRCUITS & PLASTICITY
SALVE REGINA UNIVERSITY
NEWPORT, RI
JUN 26-JUL 1, 2005
GINA TURRIGIANO, CHAIR
RAFAEL YUSTE, VICE CHAIR

- **Keynote Speaker** (Rodolfo Llinas)
- **Circuit Formation** (Holly Cline / Josh Sanes / Tom Sudhof)
- **Local Control of Synaptic Properties** (Erik Kandel / Kelsey Martin / Mike Ehlers)
- **Retina and Visual Circuitry** (Markus Meister / Peter Sterling / Axel Borst)
- **Olfaction/Gustation** (Larry Katz / Zach Mainen / Don Katz)
- **CPGs and Spinal Circuits** (Eve Marder / Ole Kiehn / Nick Spitzer)
- **Auditory System and Vocal Communication** (Constance Scharff / Michael Brainard / Asif Ghazanfar)
- **Persistant Activity and Motor Control** (Sascha Du Lac / David Tank / H. Sebastian Seung / Mike Shadlen)
- **Cortical and Hippocampal Circuitry and Plasticity** (David McCormick / Massimo Scanziani / Wendy Suzuki)

NEUROETHOLOGY:
BEHAVIOR, EVOLUTION & NEUROBIOLOGY
MAGDALEN COLLEGE
OXFORD, UK
AUG 7-12, 2005
NICHOLAS STRAUSFELD, CHAIR
CATHERINE CARR &
PAUL KATZ, CO-VICE CHAIRS

- **Opening Session** (*Nicholas Strausfeld* / *Paul Katz* / *Catharine Carr* / Edmund T. Rolls / Thomas Seeley)
- **Ecological Neuroethology** (*Eric Warrant* / *Anna Gislen* / Jochen Zeil / Peter Narins / Melissa Bateson / Caroly Shumway)
- **Evolution of Behavior** (*Kathy Rankin* / *Jonathan Bacon* / Hudson Reeve / Heather Eisthen / Bernard Crespi / Daniel Robert)
- **Emergent Behavior** (*Susan Fahrbach* / *Samuel S. Wang* / Dorothey Cheney / Fred Dyer / Archero Martinoli)
- **Navigation and Migration** (*Kathy Gotthard* / Anna Gagliardo / Thomas Collett / Kenneth Lohmann)
- **Recognition of Kin and Competitor** (*Lori Moreno* / *Brenda McCowan* / Olivier Pascalis / Elizabeth Tibbets)
- **From Animals to Robots** (*Ryohei Kanzaki* / *Hillel Chiel* / Barbara Webb / Bijan Pesaran / Charles Higgins / Roy Ritzmann)
- **Gene Expression and Behavior** (*Henrike Scholz* / *Charles Whitfield* / Hideako Takeuchi / Adi Mizrahi)

NEUROTROPHIC FACTORS
SALVE REGINA UNIVERSITY
NEWPORT, RI
JUN 19-24, 2005
BARBARA HEMPSTEAD, CHAIR
DAVID GINTY, VICE CHAIR

- **Axonal Control** (*Nancy Ip* / Stephen Strittmatter / Bill Snider / Paul Letourneau / Frank Gertler)
- **Neurotrophic Factor Signaling I** (*Mark Bothwell* / Chris Garcia / Phil Barker / Sungok Yoon / Carlos Ibanez)
- **Actions in Development I** (*Louis Reichardt* / Yves-Alain Barde / Perry Bartlett / Shahin Rafii / Luis Parada)
- **Neurotrophic Factor Signaling II** (*Bill Mobley* / Marino Zerial / Elisabeth Fisher / Roz Segal / Simon Halegoua)
- **Actions in Adulthood and Disease** (*Eric Shooter* / Valina Dawson / Mark Tuszynski / Holly Ingraham)
- **Synaptic Actions** (*Rita Balice-Gordon* / Lino Tessarollo / Bai Lu / Pietro DeCamlli)
- **Therapeutic Implications** (*Moses Chao* / Freda Miller / Dinah Sah / Jeff Milbrandt / Lieve Moons)
- **Actions in Development II** (*Carol Mason* / Susan McConnell / Kuo-Fen Lee / Anders Nykjaer)

NONLINEAR SCIENCE
COLBY COLLEGE
WATERVILLE, ME
JUN 26-JUL 1, 2005
EDWARD OTT, CHAIR
ANNA LIN, VICE CHAIR

- **Granular Media** (*Wolfgang Losert* / Harry Swinney / Robert Behringer)
- **Pattern Formation** (*Ken Showalter* / Leonard Sander / Hugues Chaté)
- **Animal Grouping** (Iain Couzin / Chad Topaz)
- **Living Systems** (Eshel Ben-Jacob / Z. Jane Wang)
- **Fluid Singularities** (Tomas Bohr / Detlef Lohse)
- **Systems and Singularities** (Martin Hasler / Shankar Venkataramani)
- **Dynamics with Lasers** (*Raj Roy* / Thomas Antonsen / Nir Davidson)
- **Turbulence** (Itamar Procaccia / Anne Juel)
- **Chaos** (*Lou Pecora* / Edward Lorenz / James Yorke)

NUCLEAR CHEMISTRY
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUN 26-JUL 1, 2005
PARTHA CHOWDHURY, CHAIR
ROY LACEY, VICE CHAIR

- **Advances in Light Nuclei**
- **Shell Structure in the 21st Century**
- **Physics Away from Stability: Neutron-Rich Nuclei**
- **Physics Away from Stability: Proton-Rich Nuclei**
- **Isomers and Nuclear Structure**
- **Symmetries, New and Old**
- **Nuclear Structure and Astrophysics**
- **Synthesis and Spectroscopy of Heavy Nuclei**
- **Weak Interactions and Nuclear Structure**
- **Exotic Excitation and Decay Modes**
- **Rare Isotope Beams and Techniques**

NUCLEAR PHYSICS
BATES COLLEGE
LEWISTON, ME
JUL 10-15, 2005
THOMAS LUDLAM, CHAIR
MICHAEL RAMSEY-MUSOLF, VICE CHAIR

- **Quark Structure of Hadrons and Nuclei**
(*Barry Holstein* / Tony Thomas / Paul Eugenio / Latifa Elouadrhiri)
- **The Spin Structure of the Nucleon**
(Werner Vogelsang / Lara De Nardo / Zein-Eddine Meziani / Steve Vigdor)
- **Phases of QCD Matter I**
(*Barbara Jacak* / Ulrich Heinz / John Harris / Larry McLerran)
- **Phases of QCD Matter II**
(Frithjof Karsch / Xin-Nian Wang / Paul Sorensen / Barbara Jacak)
- **The Structure of Hadrons**
(Betsy Beise / Rolf Ent / Barry Holstein)
- **Nuclear Structure at the Frontier**
(*Rick Casten* / Rick Casten / Witek Nazarewicz / Thomas Glasmacher / Guy Savard)
- **Nuclear Astrophysics**
(Micael Wiescher / Tony Mezzacappa / Madappa Prakash)
- **The Universe Now**
(*Larry McLaran* / Mark Trodden)
- **Nuclear Physics and the Standard Model**
(*Michael Ramsey-Musolf* / Joshua Klein / Brad Filippone / Paul Souder / Kevin Lesko)

NUCLEIC ACIDS
SALVE REGINA UNIVERSITY
NEWPORT, RI
JUN 5-10, 2005
MARTHA FEDOR & JOHN TAINER, CO-CHAIRS
STEPHEN KOWALCZYKOWSKI &
SCOTT STROBEL, CO-VICE CHAIRS

- **Chromosome Structure and Maintenance**
(*Thomas Cech* / Elizabeth Blackburn / Martin Gorovsky)
- **RNAi and MicroRNA**
(*David Bartel* / Phillip Zamore / Leemor Joshua-Tor)
- **RNA-Mediated Regulation in Bacteria**
(*Charles Yanofsky* / Robert Batey / Karen Wassarman / Andrew Feig)
- **DNA Replication and Recombination**
(*Lorena Beese* / Phoebe Rice / Stephen Benkovic / Karl-Peter Hopfner)
- **DNA Damage, At-Risk Motifs and Repair**
(*Samuel Wilson* / Cynthia Burrows / Cynthia McMurray)
- **Nucleic Acids Structure and Dynamics**
(*Carlos Bustamante* / Anna Marie Pyle / Vincent Croquette / Michelle Wang)
- **Ribosomes and Translation**
(*Matthias Hentze* / Joseph Puglisi / James Williamson / Nenad Ban)
- **Transcription and Chromatin**
(*Joan Conaway* / Karolin Luger / Richard Gourse / Jeff Gelles)
- **RNA Processing**
(*Allan Jacobson* / Holger Stark / Brent Graveley)

NUCLEOSIDES, NUCLEOTIDES & OLIGONUCLEOTIDES
SALVE REGINA UNIVERSITY
NEWPORT, RI
JUN 26-JUL 1, 2005
DONNA SHEWACH, CHAIR
JYOTI CHATTOPADHYAYA, VICE CHAIR

- **Keynote Speaker**
(William Plunkett)
- **G-quadruplex Interactions**
(*Laurence Hurley* / Stephen Neidle / Laurence Hurley)
- **DNA Replication and Fidelity**
(Thomas Kunkel / Joann Sweasy / Floyd Romesberg / Michael Famulok)
- **Innovative Nucleic Acid Tools**
(Anna Khvorova / Oliver Seitz)
- **Novel Nucleoside Analogs**
(Frank Seela / Mitsuo Sekine)
- **Nucleosides in Gene Transfer Therapy**
(Margaret Black / Barry Stoddard / Svend Freytag)
- **New Antivirals**
(*Karen Anderson*)
- **Hot Topics**

OCULOMOTOR SYSTEM BIOLOGY
BATES COLLEGE
LEWISTON, ME
JUN 26-JUL 1, 2005
LAWRENCE MAYS &
JOHN PORTER, CO-CHAIRS
NEERAJ GANDHI &
JENNIFER GROH, CO-VICE CHAIRS

- **Pulling Together the Disparate Parts of Eye Movement Control Systems**
(*David L. Sparks* / Joseph L. Demer / Stephen Lisberger / David Zee)
- **Integrative Biology of Extraocular Muscle**
(*John D. Porter* / Linda McLoon / Jean Buttner-Ennever / Stephen J. Goldberg / Henry J. Kaminski)
- **Saccade-Vergence Interaction**
(*David S. Zee* / Claudio Busettini / Michael King / R. John Leigh / Kathleen Cullen)
- **Integration of Oculomotor Subsubstems**
(*Kathleen Cullen* / J. Douglas Crawford / Edward G. Freedman / Dora E. Angelaki / Neeraj Gandhi)
- **Mechanisms of Sensorimotor Integration**
(*Neeraj Gandhi* / William C. Hall / Edward L. Keller / John Van Opstal / Raymon M. Glantz)
- **Coordinate Transformations for Action**
(*Paul Glimcher* / Carol L. Colby / Jennifer M. Groh / Terry Stanford / Luis Populin)
- **Keynote: Where Do We Go From Here?**
(*Lawrence E. Mays* / John Maunsell / David L. Sparks)
- **Deciding [Where to Look][to Act]**
(*Jennifer M. Groh* / Paul W. Glimcher / Richard J. Krauzlis / Michele Basso / Paul Gamlin)
- **New Approaches / Perspectives on Ocular Motility Disorders**
(*R. John Leigh* / Elizabeth C. Engle / John S. Stahl / Michael Mustari)

ORGANIC REACTIONS & PROCESSES
BRYANT UNIVERSITY
SMITHFIELD, RI
JUL 31-AUG 5, 2005
STEVEN KING, CHAIR
MATT MCINTOSH, VICE CHAIR

- **New Reactions in Organic Synthesis 1**
(*Anna Mapp* / Ed Vedejs / David Barnes / Sergey Kozmin)
- **Organo Metallic Chemistry in Organic Synthesis 1**
(David Collum / Viresh Rawal / Jerry Murry / Dali Sames / Shannon Stahl)
- **Recent Developments in Catalysis**
(*Margaret Hsu* / David MacMillan / Sally Gut / Brian Stolz)
- **New Reactions in Organic Synthesis 2**
(*Karl Scheidt* / Shu Kobayashi / Jonathan Ellman / Kai Rossen / Tom Rovis / Greg Dake)
- **Mechanistic Chemistry 1**
(*Rodney Parsons* / Donna Blackmond / Ed Delany)
- **Selectivity and Target Oriented Synthesis**
(*Lisa Reeder* / Steven Burke / Scott Rychnovsky / Bernhard Breit / Melanie Sanford)
- **Organo Metallic Chemistry in Organic Synthesis 2**
(Dick Schrock / Wayne Luke)
- **Mechanistic Chemistry 2**
(*Matt MacIntosh* / Daniel Singleton / Guy Lloyd-Jones / Michel Journet)
- **Special Lecture**
(*Bill Bailey* / Reinhard Hoffmann)

ORGANIC THIN FILMS
SALVE REGINA UNIVERSITY
NEWPORT, RI
JUL 10-15, 2005
NICHOLAS ABBOTT &
MILAN MRKSICH, CO-CHAIRS
MARTIEN COHEN STUART &
NICHOLAS KOTOV, CO-VICE CHAIRS

- **Monolayers**
(David Reinhoudt / Christopher Chidsey / Jan Genzer)
- **Polymer Thin Films**
(Tom Russell / Christine Luscombe / Paul Nealey / Jim Watkins)
- **Liquid Crystalline Films**
(Philippe Poulin / Peter Palffy-Muhory / Timothy Bunning)
- **Electrooptical Thin Films**
(Mary Galvin / Stephen Forrest)

- **Single Biomolecule Force Measurements**
  (Yves Dufrene / Deborah Leckband)
- **Biomolecular Interfaces**
  (Horst Vogel / Ka-Yee Lee / Christopher Lowe)
- **Scaffolds for Cells**
  (Kevin Healy / David Mooney / Philip Messersmith)

ORGANOMETALLIC CHEMISTRY
SALVE REGINA UNIVERSITY
NEWPORT, RI
JUL 10-15, 2005
PAUL FAGAN, CHAIR
GERARD PARKIN, VICE CHAIR

- **Alexander M. Cruickshank Lecturer**
  (Robert Grubbs)
- **Organometallics in Organic Synthesis**
  (Barry Trost / Greg Fu / David Teller)
- **Organometallics in Catalysis**
  (Johannes de Vries / Daniel Mindiola)
- **Synthetic/Mechanistic Organometallic Chemistry**
  (Peter Wolczanski / Paul Chirik / Vlad Grushin)
- **Organometallic Lanthanide/Actinide Chemistry**
  (Karsten Meyer)
- **Physical Methods**
  (Paul Pregosin)

ORIGINS OF SOLAR SYSTEMS
CONNECTICUT COLLEGE
NEW LONDON, CT
JUN 26-JUL 1, 2005
JOSEPH NUTH, CHAIR
LEE HARTMANN, VICE CHAIR

- **What Have We Learned from Extrasolar Planetary Systems?**
  (William Cochran / Artie Hatzes / Alan Boss)
- **The Genesis Mission: The Initial Composition of the Solar System**
  (Donald Burnett / Charles Hohenberg / Robert Pepin)
- **Spitzer Observations of Protoplanetary Disks**
  (Michael Meyers / Dan Watson / Paola D'Alessio)
- **Presolar Production of Short-Lived Radio Isotopes**
  (Katrina Lodders / Roger Chevalier / Al Cameron)
- **Local Production of Short-Lived Radio Isotopes**
  (Guy Consolmagno / Eric Feigelson / Kevin McKeegan)
- **Constraints on Nebular Processes from Presolar Grains**
  (Sara Russell / Scott Messenger / Gary Huss)
- **Disk Chemistry from Comets, Meteorites and Spitzer**
  (Geoff Blake / Paola Caselli / Bruce Fegley)
- **Disk Dynamics: The Formation and Migration of Planets**
  (Stuart Weidenschilling / Scott Kenyon / Richard Nelson)
- **Comparative Planetology: Is the Earth Unique?**
  (Geoffrey Taylor / Michael Drake)

PHAGOCYTES
CONNECTICUT COLLEGE
NEW LONDON, CT
JUN 12-17, 2005
MARY DINAUER, CHAIR
JOEL SWANSON, VICE CHAIR

- **Zanvil Cohn Lecture**
  (*Sam Silverstein*)
- **Regulation of Myelopoiesis**
  (*Robert Clark* / Stu Orkin / Dan Tenen / Dan Link / Klaus Ley)
- **Actin and Membrane Dynamics**
  (*Tom Stossel* / Fred Maxfield / Fred Southwick / Becky Worthylake / Tom Stossel)
- **Inflammation and Disease**
  (*Dirk Roos* / Dan Kastner / Lisa Coussens / John Harlan)
- **Leukocyte/Endothelial Cell Interactions**
  (*Gary Bokoch* / Claire Doerschuk / Francisco Sanchez-Madrid)
- **Innate Immunity and Toll Receptors**
  (*Alan Ezekowitz* / Bruce Beutler / Jin Mo Park / Roland Strong)
- **Phagocytosis and Host Pathogen Interactions**
  (*Siamon Gordon* / Sergio Grinstein / Steve Greenberg / Lee-Ann Allen / Rick Brown)
- **Microbial Killing**
  (*William Nauseef* / Anthony Segal / Elizabeth Ligeti / Tom DeCoursey)
- **Signal Transduction**
  (*Linda McPhail* / Edgar Pick / Michael Yaffe / Phill Hawkins / Carl Nathan)
- **Emerging Technologies**
  (*John Curnutte* / Alan Aderem / Adrian Ozinsky)

PHOTOACOUSTIC & PHOTOTHERMAL PHENOMENA
INTERNATIONAL CENTER FOR THEORETICAL PHYSICS
TRIESTE, ITALY
JUN 26-JUL 1, 2005
MLADEN FRANKO & JOSEF PELZL, CO-CHAIRS
MASAHIDE TERAZIMA, VICE CHAIR

- **Nanoscale Thermal Properties and Transport**
  (*Bernard Cretin* / Azedine Hammiche / Ludwig Balk)
- **Nanoparticles**
  (*Jan Thoen* / Egon Matijevic / B. Lounis / Vladimir Zharov)
- **Medical Applications**
  (*A.A. Oraevsky* / Werner Faubel / Lihong V. Wang)
- **Linear and Nonlinear Laser Sound**
  (*Vitaly Gussev* / Sergey V. Egerev / Peter Hess / Otto Muskens)
- **Ultrafast and Transient Phenomena**
  (*David Hurley* / M. Yamaguchi / O.B. Wright)
- **Analytical Chemistry and Photochemistry**
  (*T. Autrey* / Stephen Bialkowski / Chieu. D. Tran / Silvia Braslavsky)
- **Food Quality and Environmental Analysis**
  (*Helion Vargas* / Frans J.M. Harren / Dane Bicanic)
- **New Instrumental Developments**
  (*Mihai Chirtoc* / Frank K. Tittel / J.G. Diebold / Ralph Meckenstock)
- **Nondestructive Evaluation**
  (*Hassan Talaat* / Andreas Mandelis / Gerd Busse)

PHOTOCHEMISTRY
BRYANT UNIVERSITY
SMITHFIELD, RI
JUL 10-15, 2005
VAIDHYANATHA RAMAMURTHY &
KIRK SCHANZE, CO-CHAIRS
LINDA JOHNSTON, VICE CHAIR

- **Molecular, Supramolecular and Chiral Photochemistry**
  (N.J. Turro / M. Garcia-Gariba / J.C. Scaiano / T. Bach / B. Feringa / R.G. Weiss)
- **Materials Photochemistry**
  (L. Brus / G. Calzaferi / Anna Köhler / O. Nalamasu)
- **Bio-Photonics and Sensors**
  (D.G. Whitten / W. Tan / L. Tolbert / P. Ogilby)
- **Energy Conversion**
  (E. Galoppini / H. Arakawa)
- **Physical and Theoretical Photochemistry**
  (B. Schwartz / J. Michl / M. Robb / J. McCusker / P. Coppens)

PHOTOSYNTHESIS
BRYANT UNIVERSITY
SMITHFIELD, RI
JUL 3-8, 2005
SABEEHA MERCHANT, CHAIR
R. DAVID BRITT, VICE CHAIR

- **Structure Function Analysis**
  (*A. William Rutherford* / Kevin Redding / Bridgette Barry / Junko Yano)
- **Proteomics and Functional Genomics**
  (*Robert Blankenship* / Norbert Rolland / Dario Leister / Jeffrey Moseley / J. Thomas Beatty / Devaki Bhaya / Donald A. Bryant)
- **Proton and Electron Transfer**
  (*David Britt* / Fevzi Daldal / Wolfgang Junge / Frauke Baymann)
- **Protein Targeting and Biogenesis of Complexes**
  (*Eva-Mari Aro* / Danny Schnell / Steven Theg / Maria Ghirardi / Richard Kuras / Himadri Pakrasi)
- **CF_1 Biogenesis and Regulation and ATP Synthesis**
  (*David Kramer* / Toru Hisabori / Toshiharu Shikanai / Francis-André Wollman)
- **Pigment and Cofactor Synthesis, Assembly and Regulation**
  (*Elisabeth Gantt* / Ayumi Tanaka / Frédéric Barras / Stéphane Lobréaux / Niels-Ulrik Frigaard / Angela Falciatore)
- **Antenna Protein Structure, Function and Regulation**
  (*Richard Cogdell* / Stefan Jansson / Jean-David Rochaix / David Kehoe)
- **Redox, Environmental Control and State Transitions**
  (*Christine Foyer* / Klaus Apel / Krishna Niyogi / Aaron Kaplan / Giovanni Finazzi)
- **Structures of Photosynthetic Complexes**
  (*Marilyn Gunner* / James Barber / Nathan Nelson / Neil Hunter)

PHYSICAL ORGANIC CHEMISTRY
HOLDERNESS SCHOOL
PLYMOUTH, NH
JUN 26-JUL 1, 2005
GARY WEISMAN, CHAIR
R. STAN BROWN, VICE CHAIR

- **Stereochemistry; Industry & the Discipline**
  (*Charles Perrin* / Jay Siegel / Eusebio Juaristi / Ed Wasserman)
- **Supramolecular Chemistry**
  (*Tyler McQuade* / Cornelia Bohne / Luis Echegoyen / *Olaf Wiest* / Lyle Isaacs / Silviu Balaban)
- **Computational Chemistry**
  (*Richard Johnson* / Weston Borden / David Giesen / Martin Saunders)
- **Structure and Function I**
  (*Roger Alder* / Natia Frank / Darren Hamilton / *Lawrence Scott* / Armin de Meijere / Rik Tykwinski)
- **Organometallic Chemistry**
  (*David Lemal* / Guy Lloyd-Jones / David am Ende / Hans Reich)
- **Mechanisms, Intermediates, and Structure**
  (*Stephen Nelsen* / Edward Clennan / Steven Kass / *William Leigh* / Frances Cozens / Jonathan White)
- **Enzymes**
  (*Malcolm Forbes* / John Richard / Peter Petillo / Martin Tanner)
- **Structure and Function II**
  (*Nancy Goroff* / Joseph Lambert / Thomas Bell / *Anna Gudmundsdottir* / Kathleen Kilway / Peter Schreiner)
- **Poster Session Talks**
  (*Stan Brown*)

PLANT METABOLIC ENGINEERING
TILTON SCHOOL
TILTON, NH
JUL 10-15, 2005
ELEANORE WURTZEL, CHAIR
ERICH GROTEWOLD, VICE CHAIR

- **Plant Chemistry and Ethnobotany**
  (Jim Simon / Dennis Stevenson / Jorge Vivanco)
- **Metabolic Profiling and Spectroscopic Tools**
  (*Richard Trethewey* / Lloyd W. Sumner / Xuemin Wang / Ruth Stark)
- **Metabolic Flux Analysis and Modeling**
  (*Andrew Hanson* / David Gang / John Ohlrogge / Lukas Mueller)
- **Enzyme Structure and Assembly of Complexes**
  (*Deborah Delmer* / Birger Møller / Brenda Winkel / Joseph Noel)
- **Metabolic Compartmentalization**
  (*Virginia Walbot* / George Wagner / Peter Facchini / Enrico Martinoia)
- **From Genomes to Pathways**
  (*Joe Chappell* / Clint Chapple / Kevin Walker / Yossi Hirschberg)
- **Engineering the Senses**
  (*Gad Galili* / Eran Pichersky / Francesca Quattrocchio)
- **Plants as Chemical Factories**
  (*Dean Della Penna* / Alisdair Fernie / Jacqui Shanks / Craig Nessler)
- **Emerging Technologies: A Window to the Future**
  (Jay Keasling / Hal Alper / Bernhard Palsson)

PLASMID & CHROMOSOME DYNAMICS

(see **CHROMOSOME DYNAMICS**)

POLYAMINES
CONNECTICUT COLLEGE
NEW LONDON, CT
JUN 12-17, 2005
EUGENE GERNER &
ANTHONY MICHAEL, CO-CHAIRS
LEENA ALHONEN &
MARGARET PHILLIPS, CO-VICE CHAIRS

- **Physiological Roles**
  (*Susan Gilmour* / John Cleveland / Juahni Janne / Leonard Johnson)
- **Transport**
  (*Keiko Kashiwagi* / Takeshi Uemura / Mattias Belting / Marie-Pierre Hasne / Richard Poulin)
- **Mechanisms of Action: RNA / Translation**
  (*John Atkins* / Eric Westhof / Tairo Oshima / Kazuei Igarashi)
- **Mechanisms of Action: RNA Processing / eIF-5A**
  (*Albert Abbruzzese* / Myung Park / Sandro Valentini / Annette Kaiser)
- **Mechanisms of Action: Translational Frameshifting / Antizyme**
  (*Senya Matsufuji* / Phillip Farabaugh / Marvin Hackert / Philip Coffino)
- **Mechanisms of Action: Signaling / Transcription**
  (*J.Y. Wang* / Lisa Shantz / Ralf Baumeister / Kirsi-Marja Oksman-Caldentey)
- **Pathophysiological Functions: Cancer**
  (*Diane McClosky* / David Feith / Robert Casero / Frank Meyskens)
- **Pathophysiology / Clinical Applications**
  (*Lo Persson* / Carol Colton / Karen Doyle)
- **Debate: Therapeutic Strategies (Analogs Versus Target-Directed Drugs)**
  (*Carl Porter* / **Pro-Analogs**: Patrick Woster / Heather Wallace / Laurence Marton / **Pro Target Directed**: Ian Blagbrough / Victor Levin)

POLYMER COLLOIDS
TILTON SCHOOL
TILTON, NH
JUL 3-8, 2005
KOICHI TAKAMURA, CHAIR
ALEX VAN HERK, VICE CHAIR

- **Control in Particle Shape and Molecular Structure**
  (*Francoise Candau* / Masayoshi Okubo / Bernadette Charleux)
- **Challenge in New Industrial Processes and Products**
  (*Mamoru Nomura* / Ed Kostansek / Bedri Erdem / Dennis Larah)
- **New Development in Industrial Applications**
  (*Do Ik Lee* / Wolf-Dieter Hergeth / Charles McDonald)
- **Novel Composite Colloids**
  (*Mitchell Winnik* / Wen-Ying Chiu / Onder Pekcan / Jennifer Lewis)
- **Challenge in Process Engineering**
  (*Jose Asua* / Mike Taylor / Andrew Klein)
- **Control in Dispersion Stability**
  (*Peter Lovell* / Yilong Han / Darrell Velegol / Theodorus van de Ven)
- **Bio Medical Applications**
  (*Haruma Kawaguchi* / Yukio Nagasaki / Eugenia Kumacheva / Guanghi Ma)
- **Self-Assembly**
  (*Alex van Herk* / Younan Xia / Eric Dufresne)

POLYMERS (EAST)
MOUNT HOLYOKE COLLEGE
SOUTH HADLEY, MA
JUN 19-24, 2005
KRZYSZTOF MATYJASZEWSKI, CHAIR
KAREN WOOLEY, VICE CHAIR

- **Functional Complex Macromolecules**
  (*Tim Long* / Takuzo Aida / Jean Frechet)
- **Biological Routes to Polymers**
  (*Kristi Kiick* / Richard Gross / David Tirrell)
- **Polymeric Tissue and Drugs**
  (*Heather Maynard* / Jeff Hubbell / Ruth Duncan)
- **Molecular Brushes**
  (*William Brittain* / Christine Ortiz / Sergei Sheiko)
- **Reinventing Polyolefins**
  (*Bruce Novak* / Jim Stevens / Pete Fujita)
- **New Materials via Precise Polymer Synthesis**
  (*Tim Patten* / Yves Gnanou / Jeff Pyun)
- **Bioinspired Nanomaterials**
  (*Helmut Ringsdorf* / Sam Stupp / Virgil Percec)
- **Controlling Ionic and Radical Polymerizations**
  (*Stuart Rowan* / Stan Penczek / Mitsuo Sawamoto)
- **Polymers for Optoelectronics**
  (*Florian Schattenmann* / Chris Ober / Ian McCulloch)
- **Nanoscale Organization of Polymers**
  (*Doug Kiserow* / Frank Bates / Ned Thomas)
- **Novel Materials from Unconventional Media**
  (*Bernadette Charleux* / Joe DeSimone / Markus Antonietti)
- **Polymers at Surfaces**
  (*Martin Moeller* / Tom McCarthy / Tomek Kowalewski)
- **Macromolecular Nano-Objects**
  (*Valerie Shears* / Axel Mueller / Craig Hawker)

POLYSACCHARIDES, CHEMISTRY OF
HONG KONG UNIVERSITY OF SCIENCE & TECHNOLOGY
HONG KONG, CHINA
JUN 5-10, 2005
SHIGENORI KUGA, CHAIR
DEREK GRAY, VICE CHAIR

- **Biosynthesis and Chemical Synthesis 1**
  (*Nick C. Carpita* / Steven Ball / Vincent Bulone)
- **Biosynthesis and Chemical Synthesis 2**
  (*Vincent Bulone* / Shin-ichiro Nishimura / Nick C. Carpita / Jun-ichi Kadokawa)
- **Biodegradation and Enzymatic Treatments 1**
  (*Harry Brumer* / Masahiro Samejima / Antje Potthast)
- **Biodegradation and Enzymatic Treatments 2**
  (*Yoshiharu Nishiyama* / Takuya Kitaoka / Harry Brumer)
- **Structure and Properties**
  (*Antje Potthast* / Yoshiharu Nishiyama / John W. Brady / Stephen J. Eichhorn)

- **Complex Formation**
  (*Laurent Heux* / Alain Buleon / Shinichi Kitamura / Bjorn T. Stokke)
- **Colloids and Materials 1**
  (*Bjorn T. Stokke* / Masahisa Wada / Laurent Heux)
- **Colloids and Materials 2**
  (*Saad A. Khan* / Francois Ravenelle / Dieter Klemm / Hiroyuki Yano)
- **Colloids and Materials 3**
  (*Dieter Klemm* / Lina Zhang / Saad A. Khan)

PROTEIN TRANSPORT ACROSS CELL MEMBRANES
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUN 12-17, 2005
HARRIS BERNSTEIN &
ROSS DALBEY, CO-CHAIRS
JURGEN SOLL, VICE CHAIR

- **Protein Targeting**
  (*Lila Gierasch* / Roland Beckmann / Ralph Henry / Olaf Schneewind)
- **Protein Import into Mitochondria**
  (*Trevor Lithgow* / Toshiya Endo / Carla Koehler / Klaus Pfanner / Rosemary Stuart)
- **Translocation Across the Bacterial Inner Membrane**
  (*Tassos Economou* / Arnold Driessen / Matthias Muller / Tracy Palmer)
- **Protein Translocation Into and Out of the ER**
  (*Reid Gilmore* / Manu Hegde / Hidde Ploegh / Yihong Ye)
- **Peroxisome Protein Import**
  (*Paul Lazarow* / Ralf Erdmann / Yukio Fujiki / Steven Gould)
- **Transport to the Bacterial Cell Surface and Beyond**
  (*Hajime Tokuda* / Peter Christie / Vassilis Koronakis / Tony Pugsley / Jan Tommassen)
- **Protein Import into Chloroplasts**
  (*Colin Robinson* / Ken Cline / Danny Schnell / Steve Theg)
- **Insertion/Folding of Membranes Proteins**
  (*Gunnar von Heijne* / Bill Dowhan / Art Johnson / Andreas Kuhn / Bill Skach)
- **"Non-Classical" Mechanisms of Protein Transport Across Cell Membranes**
  (*David Andrews* / Steve Dowdy / Francoise Jacob-Dubuisson / Anna Rubartelli)

PROTEINS
HOLDERNESS SCHOOL
PLYMOUTH, NH
JUN 19-24, 2005
VIRGINIA RATH & FRANZ SCHMID, CO-CHAIRS
CHRISTOPHER HILL &
GARY PIELAK, CO-VICE CHAIRS

- **Opening Keynote Speaker**
  (*Franz Schmid* / Susan Lindquist)
- **Protein Folding and Dynamics I**
  (*Terry Oas* / George Rose / Art Palmer / Sheena Radford / Andy Robertson / Vijay Pande)
- **Protein Folding and Dynamics II**
  (*Charles Brooks* / Dan Raleigh / Rudi Glockshuber)
- **Protein Design**
  (*Donald Doyle* / Homme Hellinga / Brian Kuhlman / Lynne Regan / Steve Mayo / Andreas Pluckthun)
- **Natively Disordered Proteins**
  (*Trevor Creamer* / Peter Wright / Keith Dunker)
- **Membrane Proteins**
  (*Chris Hill* / Robert Stroud / Harvey McMahon / Lukas Tamm / Dinesh Yernool / Jim Bowie)
- **Proteins in Disease**
  (*Gary Pielak* / Ron Wetzel / Jonathan Weissman)
- **Protein Complexes and Networks**
  (*Volker Dotsch* / John Kuryian / Lila Gierasch / Art Johnson / Janet Thornton / Tania Baker)
- **Closing Keynote Speaker**
  (*Virginia Rath* / Wendell Lim)

QUANTUM CONTROL OF LIGHT AND MATTER
COLBY COLLEGE
WATERVILLE, ME
JUL 31-AUG 5, 2005
PAUL CORKUM, CHAIR
PHILIP BUCKSBAUM &
DAVID TANNOR, CO-VICE CHAIRS

- **Opening Session**
  (H. Rabitz)
- **Controllability**
  (*P. Brumer* / R. Brockett / N. Khaneja / C. Rangan / T. Weinacht)
- **The Role of De-Coherence**
  (*M. Scully* / D. Lidar / E. Shapiro)
- **Attosecond and X-Rays**
  (*S. Leone* / F. Kaertner / M. Murnane / R. Kienberger)
- **Light Matter Interaction at its Extreme**
  (J. Kimble / L. Novotny)
- **Control in Few Level Systems**
  (K. Bergmann / S. Rice)
- **Wave Packets and Their Applications**
  (*T. Kobayoshi* / R. Jones / D. Villeneuve)
- **Chemical Control**
  (*R. Levis* / N. Schwentner / V. Bonacic-Koutecky / G. Gerber / Y. Ohtsuki)
- **Quantum Thermodynamics and Cooling**
  (M. Raizen / R. Kosloff)

RADIATION & CLIMATE
COLBY COLLEGE
WATERVILLE, ME
JUL 24-29, 2005
HOWARD BARKER &
ROBERT ELLINGSON, CO-CHAIRS
WILLIAM COLLINS &
PHILIP RUSSELL, CO-VICE CHAIRS

- **Hydro-Radiative Climatology**
  (*Dennis Hartmann* / Graeme Stephens / Syukuro Manabe)
- **Absorption and Scattering**
  (*Qiang Fu* / Eli Mlawer / Klaus Pfeilsticker / Micheal Mishchenko)
- **Radiative Transfer I: Two-Stream Approximation and Averaging**
  (*James Coakley, Jr.* / Warren Wiscombe & Philip Gabriel / Alexander Marshak)
- **Radiative Transfer II: Dynamical Models**
  (*Thomas Ackerman* / Petri Räisänen / Bernard Pinty / Dana Veron)
- **Radiative Transfer III: Innovative Remote Sensing**
  (*Lee Harrison* / Qilong Min / Anthony Davis)
- **Cloud Observing Systems**
  (*Susanne Crewell* / Steven Platnick / David Turner / David Donovan)
- **Assimilation of Cloud Data**
  (*Arthur Hou* / Tomislava Vukiæeviæ / Angela Benedetti)
- **Modelling and Observing Clouds and Radiation**
  (*Wojciech Grabowski* / David Randall / Christian Jakob / Xiaoqing Wu)
- **Analyses of Cloud Feedbacks and Radiative Sensitivities**
  (*Hartmut Grassl* / Alan Betts / Bruce Wielicki)

RED CELLS
TILTON SCHOOL
TILTON, NH
JUN 12-17, 2005
LEONARD ZON, CHAIR
JON MORROW, VICE CHAIR

- **Erythroid Gene Expression: Membrane Proteins**
  (*David Bodine* / Peter Agre / Harvey Lodish / Edward Benz / David M. Bodine)
- **Erythroid Gene Expression: Globin / LCR**
  (*Douglas Engel* / Douglas R. Higgs / Frank Grosveld / Gerd Blobel / Emery Bresnik / Doug Engel / Peter Fraser)
- **Membrane Rafts, Receptors and Dynamics**
  (*Velia Fowler* / Kai Simons / Kasturi Haldar / Athar Chisti / Mohandas Narla / Rainier Prohaska)
- **RBC Structure, Organization and Volume Control**
  (*Joseph Hoffman* / Diana Gilligan / Clive Ellory / Phil Low / Ruby MacDonald / Joel Chassis / David Speicher / Stephen Goodman)
- **Transcription**
  (*James J. Bieker* / Rick Young / James J. Bieker / Nancy Speck / Patrick G. Gallagher / Joseph Prchal)
- **Red Cell Disorders**
  (*David G. Nathan* / David G. Nathan / Barry Paw / Gordon Stewart / Nancy Andrews / LuAnn Peters / Chang-Zheng Chen)
- **Stem Cells**
  (*Stuart H. Orkin* / Stuart H. Orkin / Masayuki Yamamoto / Tariq Enver / Anna Rita Migliaccio / Margaret H. Baron)
- **Hematopoiesis**
  (*Roger K. Patient* / Roger K. Patient / Stephan Karlsson / Kyunghee Choi / Bill Detrich / Toshio Suda / Elaine Dzierzak / Todd R. Evans)
- **Selected Talks from Poster Presentations**
  (*John Conboy* / *Gordon Grinder*)

SECOND MESSENGERS & PROTEIN PHOSPHORYLATION
KIMBALL UNION ACADEMY
MERIDEN, NH
JUN 12-17, 2005
JEFFREY BENOVIC &
MARK VON ZASTROW, CO-CHAIRS
NATALIE AHN &
BEVERLY ERREDE, CO-VICE CHAIRS

- **GPCR Signaling**
  (*Michel Bouvier* / Thue Schwartz / Randy Hall)
- **Regulation of GPCR / G Protein Signaling**
  (*Martin Lohse* / Joann Trejo / Maurine Linder / Heidi Hamm / Lou Luttrell)

- **Lipid Signaling**
  (*Ken Harden* / Pat Casey / Marc Lemmon)
- **Heterotrimeric G Protein Regulation**
  (*Ken Blumer* / Steve Lanier / David Siderovski / Michael Koelle / Henrik Dohlman)
- **Structural Insight into Signaling**
  (*John Sondek* / John Tesmer / Brian Kobilka / Kris Palczewski / Steve Sprang)
- **Sensory Signaling**
  (*Randy Reed* / Hiroaki Matsunami / Catherine Dulac)
- **Plenary Session**
  (*Mark von Zastrow* / Jeffrey Benovic / Charles Zuker / Robert Lefkowitz)
- **Signaling Integration / Networks**
  (*Natalie Ahn* / Rama Ranganathan / Ravi Iyengar / Alex Brown / Beverly Errede)
- **Physiological Models of Signaling**
  (*Marc Caron* / Peter Mombaerts / James Hurley / Tom Scanlan)
- **Nuclear Sensors and Signaling**
  (*Richard Fishel* / David Toczyski / Helle Ulrich / Craig Thompson)

SMALL INTEGRIN-BINDING PROTEINS
BIG SKY RESORT
BIG SKY, MT
SEP 11-16, 2005
WILLIAM BUTLER &
LARRY FISHER, CO-CHAIRS

- **Introduction to Integrins & the SIBLING Family**
  (*William Butler* / Dwayne Stupack / Tyra Wolfsberg)
- **Shared Functions of SIBLINGs**
  (*Adele Boskey* / Graeme Hunter / Vincent Castronovo / Neal Fedarko / Kalu Ogbureke)
- **Osteopontin & Mineral Metabolism**
  (*Esben Sorensen* / Cecilia Giachelli / Masaki Noda / Keith Hruska)
- **Osteopontin in Cancer, Inflammation & Immunity**
  (*Susan Rittling* / David Denhardt / Ann Chambers / Jeffrey Berman / Toshimitsu Uede / Harvey Cantor)
- **Bone Sialoprotein & Dentin Matrix Protein 1**
  (*Marc McKee* / Jaro Sodek / Jane Aubin / Anne George)
- **The CCN Family**
  (*Stephen Lam* / Lester Lau / Karen Lyons / David Brigstock)
- **DMP1 & Dentin Sialophosphoprotein**
  (*Lynda Bonewald* / Jian Feng / Mary MacDougall / Chunlin Qin)
- **DSPP, Matrix Extracellular Phosphoglycoprotein (MEPE) & Hot Topics**
  (*Michel Goldberg* / Taduru Sreenath / Peter Rowe / Thomas Brown)
- **Banquet Talk - Snake Venoms: Integrins, Disintegrins & MMPs**
  (*Larry Fisher* / Jay Fox)

STAPHYLOCOCCAL DISEASES
SALVE REGINA UNIVERSITY
NEWPORT, RI
AUG 21-26, 2005
ARNOLD BAYER & JEAN LEE, CO-CHAIRS
MATHIAS HERRMANN &
HARALD LABISCHINSKI, CO-VICE CHAIRS

- **Genomics and Proteomics under Relevant Biologic Contexts**
  (*Paul Dunman* / Ken Bayles / Mark Smeltzer / Susanne Englemann)
- **Bacterial Physiology and Metabolism Linked to Pathogenicity**
  (*Richard Proctor* / Barry Bochner / Greg Somerville / Simon Foster)
- **Regulation of Autolysis**
  (*Markus Bischoff* / Ambrose Cheung / Kelly Rice / Christiane Goerke)
- **Staphylococcal Interactions with Host Cells**
  (*Michael Yeaman* / Banhu Sinha / Timothy Foster / Hattie Gresham)
- **Immune Responses to *Staphylococcus Aureus***
  (*Andreas Peschel* / Thomas Hartung / Gabriel Nunez / Arthur Tzianabos)
- **New Concepts of Cell Wall Structure and Function**
  (*Henry Chambers* / Brigitte Berger-Bachi / Maria Pinho / Brian Wilkinson)
- **Community-Acquired Methicillin-Resistant *Staphylococcus Aureus* (CA-MRSA) - Molecular and Clinical Paradigms**
  (*Barry Kreiswirth* / Frank Lowy / Robert Daum / Frank DeLeo)
- **Issues in *Staphylococcus Epidermidis***
  (*Gordon Archer* / Michael Otto / Paul Fey / Wilma Ziebuhr)
- **Controversies/Hot Topics**
  (*Benedicte Fourniere*)

STATISTICS IN CHEMISTRY & CHEMICAL ENGINEERING
MOUNT HOLYOKE COLLEGE
SOUTH HADLEY, MA
JUL 17-22, 2005
BABATUNDE OGUNNAIKE, CHAIR
DAVID HAALAND, VICE CHAIR

- **Statistical Challenges in Systems Biology**
  (Age Smilde / *Steve Brown* / *Sijmen de Jong*)
- **Exploration and Resolution Methods Applied to Analysis of Protein Processes**
  (Anna de Juan / *Marcel Maeder*)
- **Analysis of Multivariate Images with Applications to Process Monitoring and Control**
  (John MacGregor / *Mike Piovoso* / *Jay Lee*)
- **Multi-Way Analysis of Metabonomics and NMR Data: Challenges and New Results**
  (Rasmus Bro / *Willem Windig* / *Johan Westerhuis*)
- **Applications of Statistics to Multiscale Systems**
  (Richard Braatz / *Bhavik Bakshi* / *Derrick Rollins*)
- **PharmID: Pharmacophore Identification using Gibbs Sampling**
  (Stan Young / *Aaron Owens*)
- **Comprehensive Batch Process Models with In-Situ Spectroscopic Measurements and Calorimetry**
  (Paul Gemperline / *Sara Rutan* / *Dora Kourti*)
- **Experimental Design and Analysis for Accelerated Degradation Testing of Li-Ion Cells**
  (Ed Thomas / *Brad Jones* / *Gautham Parthasarathy*)
- **Chemometrics Applications to Systems Biology: Genomics, Proteomics, Metabonomics and Lipomics**
  (Raymond Lam / *Gregory Warnes* / *David Deuwer*)

STRESS PROTEINS IN GROWTH, DEVELOPMENT & DISEASE
SALVE REGINA UNIVERSITY
NEWPORT, RI
JUL 17-22, 2005
DENNIS THIELE, CHAIR
PETER WALTER, VICE CHAIR

- **Keynote Address**
  (Elizabeth Craig)
- **Proteotoxicity**
  (Bernd Bukau / Carol Gross)
- **Stress in Cellular Compartments**
  (David Ron / Jonathan Weissman / Jeffrey Brodsky)
- **Stress Signals in Transcription Activation**
  (Lea Sistonen / Akira Nakai / Xinnian Dong / Michele Toledano)
- **Mechanisms of Stress Regulation**
  (Richard Morimoto / John Lis / Caroline Jolly / James Goodrich)
- **Stress Proteins and Survival**
  (Ivor Benjamin / Jeffrey Robbins / Kevin Morano / Doug Green)
- **Cell Biology of Stress**
  (Ron Kopito / Tso-Pang Yao)
- **Aging-Stress Axis**
  (Cynthia Kenyon / Lenny Guarente)
- **Neurodegenerative Diseases and Stress**
  (Susan Lindquist / Liming Li)

STRUCTURAL, FUNCTIONAL & EVOLUTIONARY GENOMICS
BATES COLLEGE
LEWISTON, ME
JUN 19-24, 2005
KEVIN WHITE, CHAIR
EUGENE KOONIN, VICE CHAIR

- **Functional Genomics I: Developmental Genomics**
  (*Eric Siggia* / Sean Eddy / Alan Michelson)
- **Proteins and Proteomes I: Predicting Protein Interactions**
  (*Cyrus Chothia*)
- **Functional Genomics II: Approaches to High Throughput Genetic Screening and Phenotyping**
  (*Kevin White*)
- **Evolution of Genome Complexity**
  (*Eugene Koonin* / Chris Adami / Richard Lenski / Michael Lynch)
- **Proteins and Proteomes II: Structural Genomics and Novel Protein Functions**
  (*Doug Brutlag* / Alexei Murzin / Janet Thorton / Chris Lima)
- **Proteins and Proteomes III: Macromolecular Complexes**
  (*Peer Bork*)
- **Comparative Genomics**
  (*Yoav Gilad* / Lawrence Hurst / Alex Kondrashov / Hunter Fraser)

- **Functional Genomics III: Chemical Genomics**
  (*Joel Bader*)
- **Comparative Functional Genomics**
  (*Inna Dubchak* / Arend Sidow / Adam Arkin / Dan Rokhsar)

SUPRAMOLECULES & ASSEMBLIES, CHEMISTRY OF
COLBY COLLEGE
WATERVILLE, ME
JUN 12-17, 2005
DAVID THOMPSON, CHAIR
DIRK KURTH &
JOHN TEXTER, CO-VICE CHAIRS

- **Self-Assembly Using Viral Templates**
  (Jack Johnson / Chad Mirkin)
- **Nanostructure Design and Reactivity Using Nucleic Acid-Based Templates**
  (Matt Kanan / Chengde Mao / Andy Ellington)
- **Dynamic Supramolecular Systems**
  (David Reinhoudt / Jeremy K.M. Sanders / Rint Sijbesma)
- **Molecular Recognition & Interfacial Phenomena in Crystallization**
  (Bart Kahr / Alain Brisson / Robert Tampe)
- **Hybrid Structures Emerging from Novel Amphiphilic Materials and Biomacromolecules**
  (Charles Mioskowski / Wolfgang Meier / Matt Tirrell)
- **The "Next Generation"**
  (Christine Keating / Sarah Keller / Tyler McQuade / Sergei Sheiko / Francesco Stellacci / Andrew Taton / Marcus Weck)
- **Structure & Dynamics of Phase Separated Lipid Domains in Biology**
  (Paula Booth / Nancy Thompson / Watt Webb)
- **Self-Assembly and Controlled Disassembly in Drug and Gene Delivery**
  (Frank Szoka / Henry Kopecek)
- **Biological Applications of Nanopaticles, Dendrimers & Nanogels**
  (Sönke Svenson)

THIN FILM & CRYSTAL GROWTH MECHANISMS
MOUNT HOLYOKE COLLEGE
SOUTH HADLEY, MA
JUN 26-JUL 1, 2005
MELISSA HINES, CHAIR
PETER VEKILOV, VICE CHAIR

- **Quantum Dots and Semiconductor Growth**
  (Oliver Schmidt / Vivek Shenoy / Jeff Drucker)
- **Biological Control of Crystallization**
  (Naomi Chayen / Peter L. Davies)
- **Thin Film Growth Dynamics**
  (Marcel J. Rost / Kristin Fichthorn / Jonah Erlebacher)
- **Dynamics of Crystal Growth**
  (Michael D. Ward / Miquel Salmeron)
- **Biomineralization**
  (Daniel E. Morse)
- **Control of Nucleation**
  (Bruce A. Garetz)
- **Organic Crystals**
  (Michael Doherty)
- **Hot Topics and Contributed Talks**
  (*Melissa A. Hines*)

THREE DIMENSIONAL ELECTRON MICROSCOPY
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUN 12-17, 2005
KENNETH DOWNING, CHAIR
HELEN SAIBIL &
PHOEBE STEWART, CO-VICE CHAIRS

- **Opening Session: "Whither (Molecular) Electron Microscopy?"**
  (*Kenneth Downing* / Ray Stevens / Keiichi Namba)
- **High Resolution of Structures with High Symmetry**
  (*Tim Baker* / Phoebe Stewart)
- **Better Resolution of Structures with Low Symmetry**
  (*Melissa Jurica*)
- **Molecular and Cellular Tomography**
  (*Manfred Auer*)
- **Poster Presentations and Discussion I**
  (*Teresa Ruiz*)
- **New Software Tools**
  (*Jose-Maria Carazo*)
- **Instrumentation and Specimen Preparation Advances**
  (*Bridget Carragher*)
- **Applications in Building and Refining Hybrid Structures**
  (*Chris Akey*)
- **Poster Presentations and Discussion II**
  (*Masahide Kikkawa*)

TISSUE REPAIR & REGENERATION
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUN 19-24, 2005
JUDITH ABRAHAM &
PAUL MARTIN, CO-CHAIRS
JACK GAULDIE, VICE CHAIR

- **Genetically Tractable Models of Repair**
  (*Paul Martin* / Mike Galko / Mike Redd / Howard Chang)
- **Cell Migration**
  (*Pierre Coulombe* / James Nelson / Bill Parks / Ilene Gipson)
- **Angiogenesis**
  (*Catherine Nobes* / Kairbaan Hodivala-Dilke / Ann Daugherty)
- **Inflammation**
  (*Joe Leibovich* / William Muller / Luisa DiPietro)
- **Regeneration**
  (*Ken Muneoka* / Elly Tanaka / J.C. Izpisua-Belmonte)
- **Neurorepair**
  (*Anne Logan* / Stephen Davies / Patrick Anderson / Larry Benowitz)
- **Growth Factors and Hormones in Repair**
  (*Judy Abraham* / Sabine Werner / Gillian Ashcroft / Jeff Hubbell)
- **Fibrosis**
  (*Jack Gauldie* / Mark Ferguson / Snorri Thorgeirsson)
- **Stem Cells; Clinical Applications**
  (*Paul Martin* / Richard Bucala / Yann Barrandon / Mike Longaker)

TOXICOGENOMICS
COLBY-SAWYER COLLEGE
NEW LONDON, NH
JUN 5-10, 2005
DANIEL LIEBLER &
LEONA SAMSON, CO-CHAIRS
CINDY AFSHARI, VICE CHAIR

- **Keynote Session**
  (*Leona Samson* / *Daniel Liebler* / Chris Bradfield)
- **Serum Proteomics and Markers of Toxicity and Disease**
  (*Tim Veenstra* / Daniel Chan / Robbert Slebos)
- **Toxicogenomics and Chromatin Structure**
  (*Bernard Futscher*)
- **New Technologies to Assess Protein Modifications and Toxicity**
  (*Serrine Lau* / Forrest White / Natalie Ahn / Mark Bedford / Dennis Petersen)
- **Genetic Susceptibility to Disease**
  (*Cynthia Afshari*)
- **Metabonomics to Understand Chemical Toxicity**
  (*Craig Thomas* / James Willet / John Lindon / Don Robertson)
- **Genomic Approaches to Predictive Toxicology**
  (*Ray Tennant* / James Stevens / Alison Vickers / Lisiane Meira)
- **Late-Breaking Research from Submitted Abstracts**
  (*Ben Van Houten* / *Cheryl Walker*)
- **Functional Genomics in Model Systems**
  (*Marc Vidal*)

TUBERCULOSIS DRUG DEVELOPMENT
KIMBALL UNION ACADEMY
MERIDEN, NH
JUL 3-8, 2005
JAMES SACCHETTINI, CHAIR
VALERIE MIZRAHI, VICE CHAIR

- **Opening Session**
  (*James Sacchettini* / Barry Furr)
- **Persistence Factors as Drug Targets**
  (*Eric Rubin* / David Russell / John Blanchard / Laura Via)
- **Drugs Targets and Resistance**
  (*Valerie Mizrahi* / William Jacobs)
- **High Throughput and Virtual Screening**
  (*Ken Duncan* / Tanjore Balganesh / Thomas Keller / Jose Garcia-Bustos)
- **Targeting Dormant Bacteria**
  (*Douglas Young* / John McKinney)
- **Structure-Based Approaches**
  (*Kurt Krause* / Tom Albers / Satheesh Palaninathan)
- **Lead Optimization**
  (*Melvin Spigelman* / Kim Lewis / Clifton Barry / Koen Andries)
- **Animal Models**
  (*William Bishai* / Joanne Flynn / Ann Lenaerts)
- **Clinical Trials and New Therapeutic Strategies**
  (*Denis Mitchison*)

VISUALIZATION IN SCIENCE & EDUCATION
THE QUEEN'S COLLEGE
OXFORD, UK
JUL 3-8, 2005
GEORGE LISENSKY &
PETER MAHAFFY, CO-CHAIRS
ROY TASKER &
CHRISTOPHER WATTERS, CO-VICE CHAIRS

- **Seeing and Understanding: An Overview**
  (*Mary Shultz* / Michael King / Neil Stillings)
- **Haptics and Visualization at the Micro- and Nano-Scale**
  (*Barbara Tversky* / Gail Jones / Tim Herman / Miriam Reiner)
- **Better Ways of Seeing and Understanding Science**
  (*Peter Atkins* / George Whitesides / Shaaron Ainsworth)
- **Visualizing Biological Complexity**
  (*Chris Watters* / Benno Schwikowski / Malcolm Campbell / Kathy Takayama)
- **Visualization at the Micro-and Nano-Scale: Research and Education**
  (*Zafra Lerman* / Jillian Buriak / Patti Schank)
- **Seeing and Understanding with New Tools and Environments**
  (Pat Hanrahan / Niescja Turner / Ruth Chabay)
- **Best Practices in Visualization: Pedagogical and Cognitive Perspectives**
  (*Loretta Jones* / Roy Tasker / John Moore / John Gilbert)
- **Seeing and Understanding our World and Universe**
  (*Henny Kramers-Pals* / David Uttal / Rosalind Grymes)

VITAMIN B_{12} & CORPHINS
THE QUEEN'S COLLEGE
OXFORD, UK
SEP 18-23, 2005
BERNARD GOLDING, CHAIR
WILFRED VAN DER DONK, VICE CHAIR

- **Cobalamin in Context**
  (Ebba Nexø / Wolfgang Buckel / Tetsuo Toraya)
- **B_{12} in Medicine and Toxicology I**
  (Margareta Törnqvist / William Watson / David Smith / Sergey Fedosov)
- **Protein Crystallography**
  (Christoph Kratky / Catherine Drennan)
- **B_{12} and B_{12}-Like Mechanisms**
  (Martin Newcomb / Neil Marsh)
- **B_{12} in Medicine and Toxicology II**
  (R. Carmel / Robert Kadner / Douglas Collins)
- **Cobalamin**
  (Gabi Diekert / Christoph Holliger / Rowena Matthews / Rolf Thauer)
- **Corphins**
  (Bernhard Jaun)
- **Model Systems (Chemical and Computational)**
  (Leo Radom / Rudi van Eldik / Kurt Warnke / Yoshio Hisaeda)
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  (*Gerrit van der Laan* / Peter Abbamonte)
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  (*Ahmad Moini* / Peter Smirniotis / CY Chen)
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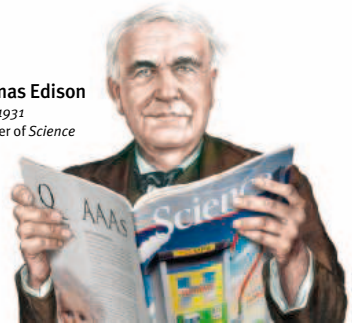

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POSITIONS OPEN

The University of Connecticut School of Dental Medicine is seeking an outstanding academic leader to direct its newly formed research Center for Regenerative Medicine and skeletal Development. The **DIRECTOR** is expected to develop a Center that will be recognized as a worldwide leader in research in the rehabilitation and regeneration of craniofacial tissue from basic, translational to clinical research. The focus of the established faculty in the Center spans the molecular regulation of skeletal and craniofacial development, the creation of biomaterials suitable for craniofacial and skeletal repair and regeneration, and the translational and clinical use of emerging approaches to restorative, prosthetic, and reconstructive dentistry.

The person selected for this position will interact with an established, highly productive, and multidisciplinary skeletal and craniofacial biology program at the University of Connecticut Health Center (UCHC), consisting of 15 investigators in the School of Medicine and 11 in the School of Dental Medicine. A generous startup package will be available as well as considerable contemporary research facilities. Two additional vacant positions also are part of the program to build a critical mass of researchers in tissue repair and regeneration.

The ideal candidate should be a strong leader with research emphasis in any of the following areas: craniofacial biology, the design of scaffolds for tissue engineering, craniofacial translational research, or the testing of experimental procedures in animals or patients, along with a solid record in grant support and publications and extensive mentoring skills.

Interested candidates should send a letter of interest and curriculum vitae to:

Dr. S. Reisine, Chair of the
School of Dental Medicine
University of Connecticut
263 Farmington Avenue, MC3910
Farmington, CT 06030

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FACULTY POSITIONS:
ASSISTANT PROFESSOR
(Tenure Track/Ph.D. Required)
LECTURER
(Nontenure Track/
Master's Degree Required)
Coastal Carolina University

The successful candidates' primary responsibility will be teaching/coordinating an interdisciplinary science course for nonmajors, but teaching assignments in the individuals' disciplines are possible. We are especially interested in applicants with a demonstrated interest in innovative methods of science education and who will collaborate with faculty from the Sciences and other Colleges within the University to further define and develop the direction of this program. While the position will be housed in the Department of Chemistry and Physics, applicants with a background in any natural science will be considered.

Applicants should submit a letter of interest, curriculum vitae, and contact information for three references to: **Louis E. Keiner, University Hall 207, Department of Chemistry and Physics, Coastal Carolina University, P.O. Box 261954, Conway, SC 29528-6054.** Review of candidates will begin on March 1, 2005, and continue until the positions are filled.

Coastal Carolina University is a growing, state-supported liberal arts institution where the emphasis is on undergraduate education, and growing importance is placed on faculty mentored student research projects and public services. Coastal Carolina University is located approximately nine miles from Myrtle Beach, South Carolina, and enrolls more than 7,000 students. *Coastal Carolina University is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

FACULTY POSITIONS in bioinformatics, medical image analysis, and visualization.

The Department of Biomedical Informatics (BMI) of the Ohio State University College of Medicine and Public Health (**website: http://bmi.osu.edu/**) seeks applications for tenure-track faculty positions at all levels.

We seek broadly trained scientists with computational research programs of biomedical relevance and collaborative potential. Scientists with research complementing current efforts are of particular interest.

Interests within BMI include: biomedical image processing and quantification, comparative genomics, phylogenetics, pharmacogenomics, cancer, protein structure, promoter and chromatin analysis, and high performance and grid computing. BMI has strong collaborations in radiology, pathology, cardiology, cancer, and automatic microscopic imaging. BMI offer opportunities to work in a rich multidisciplinary environment.

Applicants must have a proven record of publications in leading peer-reviewed journals and demonstrated potential to obtain extramural funding. Senior-level applicants (**ASSOCIATE PROFESSOR** and above) should have extramural funding. Teaching duties will include a course in an area of specialty.

Applicants should send curriculum vitae, brief statements of research and teaching interests, copies of representative publications, and have four letters of reference sent to: **Search Committee, Biomedical Informatics, The Ohio State University, 3184 Graves Hall, 333 W. 10th Avenue, Columbus, OH 43210.**

Recruitment is open immediately and will be ongoing until positions are filled.

DEPARTMENT CHAIR
Molecular, Cellular, and
Developmental Biology
University of Colorado at Boulder

We seek an internationally recognized scientist with a highly regarded research program and a commitment to graduate and undergraduate education. Molecular, Cellular, and Developmental Biology (MCDB), **website: http://mcdb.colorado.edu/**, is a distinguished and diverse department with a current faculty of 30. Attractions of this position include the expectation that during the next few years, substantial new departmental resources will flow from a drug developed using the SELEX process invented in MCDB and recently approved by the FDA. In addition the University has embarked on a parallel Biotech Initiative that will bring several new scientists to the Boulder community. The new chair will have an unusual opportunity to implement a vision of the future for MCD Biology in an outstanding academic environment.

Appointment could begin as early as fall 2005. Please send curriculum vitae, description of research plans, and the names of three references by mail to: **Jane Richards, University of Colorado, 347 UCB, Boulder, CO 80309-0347, U.S.A.,** or as PDF files by **e-mail: jane.richards@colorado.edu.** If sending by mail, please include PDF files on disk in addition to printed originals. Dossiers will be considered upon receipt until position is filled.

The University of Colorado at Boulder is committed to diversity and equality in education and employment.

RESEARCH ASSISTANT PROFESSOR. The Department of Physical Medicine and Rehabilitation, University of Kentucky College of Medicine is seeking to fill a Research Assistant Professor position in the area of spinal cord injury. Successful candidates are expected to develop and maintain innovative, nationally recognized research programs. Interested applicants should submit curriculum vitae, statement of research interests, and contact information for three references to: **Joe E. Springer, Ph.D., Physical Medicine and Rehabilitation, University of Kentucky College of Medicine, Lexington, KY 40536-0284; e-mail: jspring@uky.edu.** *Female and minority candidates are encouraged to apply. The University of Kentucky is an Equal Opportunity University.*

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ASSOCIATE II, RESEARCH

BS or MS level synthetic organic chemist required for the discovery and development of novel compounds for new therapeutic applications. Position involves synthesis of novel organic compounds and development of new synthetic methodologies. Requires BS or MS in Organic Chemistry. Proficiency with operation of common laboratory and analytical equipment and ability to interpret NMR, IR, GC, HPLC and MS reports is essential. **44002997-RK**

ASSOCIATE DIRECTOR, RESEARCH

Currently seeking PhD medicinal chemist responsible for directing a team of PhD and BS/MS level chemists performing research and development of small molecule therapeutics in a highly collaborative multi-disciplinary team environment. Experience in the design of therapeutics for oncology indications and in structure-guided drug design a plus. Requires PhD in Organic or Medicinal Chemistry with 8+ years of small molecule medicinal chemistry drug discovery experience. **44002996-RK**

DIRECTOR, RESEARCH

Candidate will lead projects in oncology from the antibody discovery phase up to IND-enabling studies. Requirements for this position include MD/PhD in Immunology or Oncology, along with 10 years of post-graduate experience, including 5 years in biotech or pharmaceuticals. **44002909-RK**

PRINCIPAL SCIENTIST, RESEARCH

Support oncology drug discovery and development. Successful candidate will sit on late stage research and early development project teams for small molecule oncology drugs. Design, implement, analyze and interpret toxicology studies in-house and at CROs, write and review regulatory documents, and sit on project teams. Experience with in vitro toxicity screening useful. Requires PhD in Toxicology or related field with 5-8 years pharmaceutical industry experience. American Board of Toxicology certification desirable. **44003008-RK**

PRINCIPAL SCIENTIST, RESEARCH

Play a central role in target validation and the preclinical evaluation of antibody therapeutics for cancer. Design, plan and perform experiments to validate targets for anti-cancer therapeutic antibodies. Requires PhD in Cell Biology or related field with more than 5 years of postgraduate experience in cancer cell biology and antibody therapeutics. **44003039-RK**

PRINCIPAL SCIENTIST, RESEARCH

Identify and develop clinically relevant biomarkers to facilitate oncological drug development and the design and execution of clinical trials, providing expertise in preclinical and clinical biomarker discovery and assay development. A PhD and 5 years post graduate experience required, along with a proven record of accomplishments. **47001425-RK**

SCIENTIST II, RESEARCH

Participate in and perform the design, development, execution, and interpretation of scientific research projects pertaining to the bioassays department. Contribute to small molecule drug discovery in the field of Oncology. Requires PhD in Applied Cell Biology or Biochemistry, with postdoctoral work and 3-5 years of related experience. **44003011-RK**

SPECIALIST II, RESEARCH

Participate in compound dispensing, replicate production, and quality control issues related to compound sample management, and support the drug discovery process within the Small Molecule Discovery division. Requires BS or MS level associate with a degree in Chemistry. Industrial experience of 2 to 5 years in a laboratory production or instrumentation setting is essential, along with familiarity with robotic automation. **44003201-RK**

SPECIALIST I, RESEARCH

Develop and perform cell-based assays as part of the hit evaluation. Employ a variety of automation instruments, signal detection technologies, and analysis techniques in this process. Ideal candidate would have experience with FACS and High-Throughput Imaging Instruments and familiarity with spreadsheet/curve-fit/graphic software. Requires BS or MS in Cell Biology, Biochemistry or related field, with minimum of 5 years work experience. **44003012-RK**

SPECIALIST I, RESEARCH

Develop and utilize in vivo models and cell-based assays to assess the efficacy and mechanism of action of novel therapeutics for oncology. Also assist in the design, development and execution of studies, analyze data, and prepare summary reports. A BS with at least at least five years of related experience in a research setting, or an MS with three years of experience is required, along with 3 years of in vivo model experience. **4003105-RK**

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Boehringer Ingelheim, ranking among the 20 leading pharmaceutical companies worldwide, is a research-driven group dedicated to researching, developing, manufacturing and marketing pharmaceuticals that improve health and quality of life.

Boehringer Ingelheim Austria in Vienna, Austria, is home to the corporation's dedicated drug discovery center for oncology. Close to 200 scientists, technicians and support staff drive our efforts to identify innovative cancer medicines. We currently have the following opening within our Department Pharmacology:

GROUP LEADER MONOCLONAL ANTIBODY DRUG DISCOVERY IN ONCOLOGY (ASSOCIATE DIRECTOR, PHARMACOLOGY)

We are looking for a highly motivated individual, with a background preferably in biology or medicine, post-doctoral experience in cancer research and several years of NBE drug discovery experience in the pharmaceutical or biotech industry, preferably in the area of monoclonal antibody therapeutics. Reporting to the Director of Pharmacology, you will lead a research group consisting of 4-5 laboratories, head a research laboratory and act as a project champion or member of multidisciplinary project teams.

Your responsibilities will include:

- contributions to target selection and target validation,
- design and implementation of antibody discovery projects,
- generation of human monoclonal antibodies using state-of-the-art technologies (phage display, transgenic mice)
- pharmacological profiling of antibody drug candidates in vitro and in animal models.

In addition, you will contribute to evaluation of in-licensing opportunities for NBE targets and drug candidates as well as NBE technologies. Excellent communication and presentation skills are required and a basic knowledge of the German language would be of advantage. Informal inquires may be directed to Dr. Günther Adolf (telephone +43 1 80105 2363, e-mail Guenther.Adolf@vie.boehringer-ingelheim.com). To formally apply for this position, please send your CV, a statement of research experience, a list of publications and potential references, quoting our reference number 117/426 to:

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CHAIR
Department of Pharmacology
Case Western Reserve University
School of Medicine

Nominations or applications are invited from established, dynamic scientists with a creative vision for the position of Chair of the Department of Pharmacology at the Case Western Reserve University School of Medicine.

The Department has great tradition, and it has interactive faculty, outstanding graduate programs, facilities, space and scientific and educational activities. With a new leadership in the School of Medicine, the Department has been targeted for significant growth to become one of the leading departments of Pharmacology in the country.

The successful candidate should have an outstanding record of scholarly achievement and a record of leadership, mentoring and administrative abilities and a commitment to expand and enhance the Department to a nationally prominent role.

Applicants should submit a letter and a statement of their research, teaching, service and administrative experience, as well as their previous mentoring experience; their legacy in their own institution in building interdisciplinary programs and resources; a C.V. and a list of publications.

Nominations and/or applications should be e-mailed to **chairphrm@cwru.edu**. For additional information, visit **http://pharmacology.cwru.edu/**. For questions or additional information you may call **Toni Scarpa** at **216-368-5298**.

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The Crop Diseases, Pests and Genetics Unit at the San Joaquin Valley Agricultural Sciences Center in Parlier, California is seeking a permanent, full-time Research Leader to: lead a multi-disciplinary team of scientists to determine the epidemiology of and develop strategies to manage, diseases caused by invasive plant pests (including plant pathogens and insects). The research will initially be focused on Xylella fastidiosa (Xf) strains and its insect vectors, especially the glassy-winged sharpshooter (GWSS).

For more details and application directions, see **www.afm.ars.usda.gov/divisions/hrd/index.html** Announcement number is **ARS-X5W-0152**. Announcement closes March 11, 2005. For questions you may contact **Dr. Ed Civerolo** on **559-596-2702** or e-mail: **eciverolo@fresno.ars.usda.gov**. U.S. Citizenship is required.

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DEPUTY MANAGER, EDUCATION AND SCIENCE & TECHNOLOGY, SUSTAINABLE DEVELOPMENT DEPARTMENT

The IDB is moving to strengthen significantly its operations aimed at supporting the development of national systems of innovation in the region.

In doing this, it draws from a long tradition of support for research, graduate education and technological development through projects that have benefited numerous government institutions, business and universities across Latin America and the Caribbean.

In today's global, knowledge based economy, science and technology have become, more than ever before, critical to economic growth, competitiveness and quality of life.

To ensure strong economic growth and reduction of poverty in the future, the region must significantly strengthen its performance with respect to all aspects of the innovation system.

The **Inter-American Development Bank**, the largest and leading source of financing for regional development in Latin America and the Caribbean, based in Washington D.C., invites applications for the new position of Deputy Manager for Education and Science & Technology, in the Sustainable Development Department.

The Deputy Manager for Education and Science & Technology is a senior member of the management team of the Sustainable Development Department with main responsibility for providing leadership and effective management in the areas of education, science & technology, including information and communication technology from the vantage point of an Institution with the mandate and resources to develop relevant projects and initiatives.

The Deputy Manager for Education and Science & Technology will:

- Oversee and coordinate technical support for operations in the areas of science and technology development, information and communication technologies for development and education. In accomplishing these tasks, the Deputy Manager will focus on working in close coordination with the Departments of the Bank directly responsible for lending and regional cooperation aimed at maximizing development effectiveness in IDB operations.
- Provide leadership within the organization to enhance and mainstream innovative policies as a key component of national development agendas and IDB financial and technical assistance activities.
- Be a focal point for the development of strategies, policies, operational guidelines and applied research agendas of the IDB and play a leading role in their dissemination and implementation both within the Bank and in the context of larger policy dialogue sustained with governments, the private sector, civil society and academia.
- Interface with IDB's partners, clients and stakeholders to promote coherence and effectiveness in international assistance to scientific, technological and educational development as well as to project the Bank's leadership in these areas.

To qualify, the applicant should have:

- A Ph.D. in science, engineering or social sciences.
- Familiarity with and a record of achievement in issues of scientific and technological development, as linked with education, the economics of innovation, competitiveness and productivity growth.
- Proven abilities as a leader and manager of a creative and highly trained team.
- Demonstrated ability to provide intellectual leadership and strategic direction.
- Strong interpersonal and communication skills.
- At least 15 years of experience (including significant international experience) in positions of increasing responsibility in the management of government, business, academic or development organizations.
- Proficiency in English and Spanish required. Working knowledge of Portuguese and/or French is desirable.

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Inter-American Development Bank

STANFORD UNIVERSITY

PALEOBIOLOGY

The Department of Geological and Environmental Sciences at Stanford University invites applications for a tenure-track faculty appointment at the Assistant Professor level in the area of Paleobiology. We are looking for a person with a demonstrated research record and who is committed to quality undergraduate and graduate teaching. While we will consider applications from individuals in all areas of paleobiology, emphasis will be placed on candidates who have an understanding of the broad evolution of life on Earth and research experience in one or more of the following areas: (1) the origin and early evolution of life, (2) relationships between the evolution of life and the major physical processes and events in Earth history, (3) external driving forces of major biological extinctions and radiations, and (4) climatic-ocean history and the evolution of the ancient marine biota. Interdisciplinary approaches are of special interest, but applicants must have a clear grounding in geology. We are seeking an individual who applies fundamental biological and geological principles, quantitative data, and field-based studies to characterize and model biological evolution.

Although the position will remain open until filled, applications, including a curriculum vitae, a statement outlining research and teaching interests that would materially contribute to related programs in the School of Earth Sciences, and the names and addresses of three referees, should be sent by **April 1, 2005**, to: **Paleobiology Search Committee, Department of Geological and Environmental Sciences, Stanford University, Stanford, CA 94305-2210.** Questions can be directed to **Prof. Donald R. Lowe (lowe@pangea.stanford.edu)** or **Prof. Stephan A. Graham (graham@pangea.stanford.edu)**.

Stanford University has a strong institutional commitment to the principle of diversity. In that spirit, we particularly encourage applications from women, members of ethnic minorities, and individuals with disabilities. http://pangea.stanford.edu/.

Faculty Position
Marine or Environmental Microbiology
University of California, San Diego
Department of Molecular Biology and Scripps Institution of Oceanography
http://biology.ucsd.edu/
http://www-sio.ucsd.edu/

The Scripps Institution of Oceanography and the Section of Molecular Biology in the Division of Biological Sciences, University of California, San Diego, invite applications for an Assistant (tenure-track), Associate (tenured), or Full Professor (tenured) in the area of marine or environmental microbiology. The successful candidate will hold joint appointments in the two departments as part of a developing program in microbiology. Minorities and women are encouraged to apply.

The successful candidate will use molecular approaches to the study of fundamental problems in prokaryotic organisms, preferably from a marine environment. Areas of interest include but are not limited to: Archaeal biology, microbial interactions in biofilms, symbioses, or pathogenesis, and genomic approaches to microbial evolution and diversity. The successful candidate is expected to develop or continue a vigorous research program of relevance to the goals of both departments and to participate in undergraduate and graduate teaching in microbiology. Salary will be commensurate with level of appointment and based on the UC salary scale.

Complete applications received by **March 15, 2005** will be assured of consideration. Applicants should send a curriculum vitae, publication list, synopsis of research interests and professional goals, and three letters of reference (forwarded separately) to: **Marine/Environmental Microbiology Search Committee, c/o Ria del Rosario, Division of Biological Sciences, 9500 Gilman Dr. 0366-A, La Jolla, CA 92093-0366**.

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UIC University of Illinois at Chicago

COLLEGE OF MEDICINE FACULTY POSITIONS

CENTER FOR IMMUNOLOGY

The Center For Immunology at the University of Illinois College of Medicine is inviting applications from individuals with a research focus in areas revolving around the central theme of immune tolerance such as Cancer Immunology, Persistent Infections, Cell/Organ Transplantation, Autoimmunity and Basic Immunology. The center will be housed in a new College of Medicine Research Building.

We are recruiting PhDs, MDs or individuals with an equivalent doctoral degree to fill a number of tenured / tenure track faculty positions at all academic ranks. Successful faculty candidates will be expected to have and maintain a vigorous independent research program as evidenced by extramural funding and consistent scholarly publications. Individuals will have primary appointments in an appropriate Basic Science or Clinical department. Generous laboratory space and start up funds are available.

For fullest consideration, please send applications, including CV, a brief statement of current research activities and future research plans and a list of 3 references to: **Search Committee, Center for Immunology, University of Illinois College of Medicine, 835 S. Wolcott (MC 790), Chicago, IL 60612-7344.**

For more information about the UIC Immunology Center, please visit our Web Site: **http://www.uicimmunology.org**.

VIROLOGY

The Department of Microbiology and Immunology in the College of Medicine at the University of Illinois at Chicago is inviting applications from individuals with a research focus in Virology revolving around viral replication, virus-host interaction, oncogenesis, pathogenesis and immunity. We are recruiting individuals with a PhD, MD or equivalent doctoral degree to tenured/tenure track faculty positions at all academic ranks. Successful faculty candidates are expected to have a vigorous research program as evidenced by extramural funding and consistent scholarly publications. The faculty of the Department of Microbiology and Immunology has active and interdisciplinary research programs spanning several major disciplines, including immunology, virology, microbial pathogenesis, host-pathogen interactions, and structural biology.

Please send applications with CV, a statement of proposed research program and a list of three references to: **Virology Search Committee, Department of Microbiology and Immunology, University of Illinois at Chicago, College of Medicine, 835 S. Wolcott (M/C 790), Chicago, IL 60612-7344.**

For more information about the Department of Microbiology and Immunology, please visit our Web Site: **http://www.uic.edu/depts/mcmi/**.

MICROBIAL PATHOGENESIS PROGRAM

The College of Medicine at the University of Illinois at Chicago is expanding its faculty in the area of Microbial Pathogenesis and is initiating an extramural search for full-time, tenured/tenure-track, faculty. Successful candidates will be appointed either in the Department of Microbiology & Immunology or the Divisions of Infectious Diseases or Gastroenterology in the Department of Medicine and most will have joint appointments. Investigators in these units along with faculty in the Department of Biological Sciences, the College of Dentistry, the College of Pharmacy and the School of Public Health provide a highly interactive research community.

We are seeking applicants at the **ASSISTANT, ASSOCIATE** or **FULL PROFESSOR** levels. Applicants must hold a PhD and/or MD degree with a proven track record in research as evidenced by consistent scholarly publications and extramural funding.

Please send applications with CV, a statement of proposed research program and a list of three references to: **Dr. Linda J. Kenney, Chair, Search Committee, Department of Microbiology and Immunology, University of Illinois at Chicago, 835 S. Wolcott, (M/C 790), Chicago, IL 60612-7344.**

INFECTIOUS DISEASES AND IMMUNOLOGY

The Infectious Diseases Section in the Department of Medicine at the University of Illinois in Chicago is recruiting research faculty to fill tenure track positions. This recruitment is being done jointly with the Department of Microbiology-Immunology and Center of Immunology at UIC to expand multidisciplinary research between these programs. Faculty candidates will have MD, MD-PhD or PhD degrees and sufficient experience to maintain independent, extramurally funded research programs. PhD candidates will have established extramural funding. ID Section faculty have existing programs that include studies of anthrax pathogenesis, viral oncogenesis, cryptococcal virulence and HIV pathogenesis and clinical trials. Joint initiatives with the Immunology Center include studies of viral oncogenesis and host response to infections.

For fullest consideration, please send an application, including CV, statement of research plans and 3 references to: **Faculty Search Committee, ID Section (MC735), University of Illinois at Chicago, 808 S. Wood Street, Chicago, IL 60612.**

For more information about the Section of Infectious Diseases, please visit our Web Site: **http://www.uic.edu/com/dom/id/.**

Review of applications for the above positions will begin **April 1, 2005**.

The University of Illinois at Chicago is an Affirmative Action/Equal Opportunity Employer.
Women and Minorities are strongly encouraged to apply.

Assistant/Associate Professor

The Department of Materials Science and Engineering at MIT invites applications for a tenure-track faculty position at the assistant/associate professor level, to begin September 2005.

Applicants should hold a Ph.D. in Materials Science and Engineering or a related science or engineering discipline. The successful candidate will be expected to develop a vibrant research program at the forefront of the field, and to harness their expertise in curriculum development and teaching at the undergraduate and graduate levels. Research areas of interest include, but are not limited to: materials chemistry, green processes, sustainable growth technologies (such as energy production), combinatorial materials characterization, material interfaces, quantum processes in materials, soft materials modeling, clinical biomaterials, synthetic biology and nanostructured materials.

Applications should be submitted with two copies of the following: a complete cv, statement of research and teaching interests, no more than three publications, and complete contact information for three references. Applications should be addressed to:

Department of Materials Science and Engineering
Att: Esther Greaves Estwick, Rm 8-328
Massachusetts Institute of Technology
77 Massachusetts Ave.
Cambridge, MA 02139-4307

Applications received by March 1, 2005 will receive full consideration. M.I.T. has a strong and continued commitment to diversity in engineering education, research and practice, and especially encourages applications from women and minorities.

Massachusetts Institute of Technology

web.mit.edu/hr

University of Zurich

The SystemsX initiative aims to place Switzerland among the leaders in systems biology and functional genomics. As part of this initiative, the Faculty of Science of the University of Zurich is seeking to fill the position of a non-tenured

Assistant Professor in Systems Biology

We are searching for outstanding individuals with a background in life sciences and a track record in the development of new approaches to systems biology and their application in the study of signal transduction, cell biology, or developmental biology. The successful candidate will be expected to establish an independent research group within the Institute of Molecular Biology. The University of Zurich will provide a competitive start-up package, state-of-the-art research facilities, and access to the Functional Genomics Center Zurich (www.fgcz.ch). There are excellent opportunities for interactions with other groups of the University of Zurich and the Swiss Federal Institute of Technology (ETH), as well as the National Centers for Competence in Research (www.snf.ch/en/rep/nat/nat_ccr_pro.asp).

The position is initially limited to three years with the possibility of renewal for another three years. Applications including a detailed curriculum vitae, publications list, short statement of research interests and the names and addresses of three referees should be submitted before March 31, 2005 to Prof. P. Truöl, Dean, Faculty of Science (MNF), University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. The application material should also be submitted as a single Word- or PDF-file to dekanat@mnf.unizh.ch.

The University of Zurich is an equal opportunity employer. Applications from women candidates are particularly encouraged.

Department of Health and Human Services
National Institutes of Health
National Institute of Nursing Research

Scientific Director
Division of Intramural Research

With nation-wide responsibility for improving the health and well being of all Americans, the Department of Health and Human Services oversees the biomedical research programs of the National Institutes of Health and those of NIH's research Institutes.

The National Institute of Nursing Research, a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services, is recruiting for a Scientific Director, a senior scientific position to lead its intramural bio-behavioral research program. The successful applicant will lead a developing group of independent principal investigators conducting research in the basic, behavioral and clinical sciences relevant to the Institute's mission (see **http://ninr.nih.gov/ninr/**) and help chart the future of a vibrant, growing nursing and bio-behavioral research community across the NIH. The Scientific Director is expected to establish a cutting edge intramural program of investigation into applying fundamental findings to bio-behavioral mechanisms. The successful candidate will be responsible for allocation of the research budget to intramural laboratory and clinical programs in coordination with rigorous, quadrennial reviews by the Board of Scientific Counselors. The Scientific Director will also be provided with a fully equipped new laboratory, supported by resources that commensurate with the size and scope of the program and will have the potential for recruitment of additional tenure-track investigators.

The successful candidate will have an earned doctorate in nursing or other health-related discipline, and an established record of outstanding research accomplishments, management of research enterprises/programs, interdisciplinary collaboration, and scientific leadership and service within the nursing and/or bio-behavioral community. Applicants should send a cover letter, curriculum vitae, detailed statement of research interests and selected publications to: **Dan Longo, MD, Co-Chair, NINR Search, c/o Dr. Melinda Tinkle, 31 Center Drive, MSC2178, Building 31/Room 5B-25, Bethesda, MD 20892-2178; tinklem@mail.nih.gov**. Applications should be received by **March 31, 2005**.

DHHS and NIH are
Equal Opportunity Employers.

Computational Chemistry and Biology Opportunities at D. E. Shaw Research and Development

Extraordinarily gifted computational chemists, biologists, and other computational scientists are sought to join a rapidly growing New York-based research group that is pursuing an ambitious, long-term strategy aimed at fundamentally transforming the process of drug discovery.

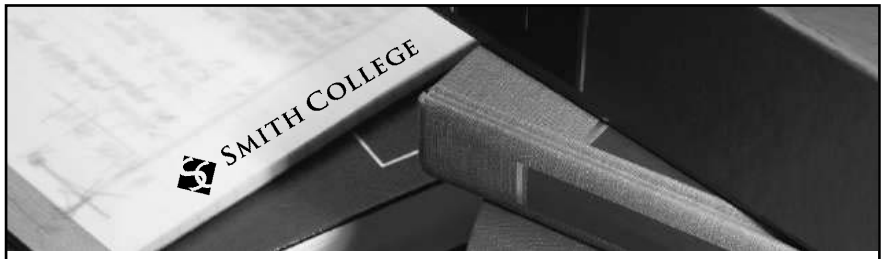
Candidates should have world-class credentials in computational chemistry, biology, or physics, or in a relevant area of computer science or applied mathematics, and must have unusually strong research skills. Relevant areas of experience might include protein structure prediction, the computation of protein-ligand binding affinities, the study of biologically important systems using molecular dynamics and/or Monte Carlo simulation, and the application of statistical mechanics to biomolecular systems—but specific knowledge of any of these areas is less critical than exceptional intellectual ability and a demonstrated track record of achievement. Current areas of interest within the group include molecular dynamics simulation of functionally significant globular and membrane proteins, the prediction of protein structures and binding free energies, structure- and ligand-based drug design, characterization of protein-protein, protein-nucleic acid and protein-lipid interactions, and the development of algorithms for biomolecular simulations.

This research effort is being financed by the D. E. Shaw group, an investment and technology development firm with more than $10 billion in aggregate capital. The project was initiated by the firm's founder, Dr. David E. Shaw, and operates under his direct scientific leadership.

We are eager to add both senior- and junior-level members to our world-class team, and are prepared to offer above-market compensation to candidates of truly exceptional ability. Please send your CV (including list of publications, thesis topic, and advisor, if applicable) to sciencemag@desrad.deshaw.com.

D. E. Shaw Research and Development, L.L.C. does not discriminate in employment matters on the basis of race, color, religion, gender, national origin, age, military service eligibility, veteran status, sexual orientation, marital status, disability, or any other protected class.

D E Shaw & Co



CLARK SCIENCE CENTER

Instrumentation and Techniques Instructor
(Half-time, 3 year term, renewable) Center for Biochemistry

Qualifications: Ph.D. in Biochemistry or related discipline. In addition, at least one year of work experience in biochemistry or related fields including proteomics is desirable. Broad expertise in biochemistry and in 2-D gel electrophoresis and LC/MS is required; expertise in spectrophotometry, spectrofluorimetry, high pressure liquid chromatography, and/or capillary electrophoresis is desirable.

Instrumentation and Techniques Instructor
(3 year term, renewable) Center for Molecular Biosciences

Qualifications: Ph.D. in Molecular Biology. In addition, at least one year of post-doctoral experience in molecular biology and bioinformatics including advanced DNA sequence and quantitative PCR analysis. Broad expertise in most areas of molecular biology including genomic and cDNA library construction, PCR, Southern and Northern blot analysis, DNA and RNA isolation, DNA fingerprinting, DNA sequence analysis, and quantitative PCR and RT-PCR, and bioinformatics. Specific experience with Applied Biosystems instrumentation preferred.

Review of resumes will begin immediately. Please forward resume, cover letter and three letters of recommendation, indicating position of interest to: **I & T Instructors Search, Smith College, Clark Science Center, Box 685, 117 Burton Hall, Northampton, MA 01063.**

To view full job descriptions, please visit our website at:
www.smith.edu/hr/career_external.php

SMITH COLLEGE

Smith College is an equal opportunity employer encouraging excellence through diversity.

Faculty Positions
Stem Cell Biology
University of California San Diego
Division of Biological Sciences
http://biology.ucsd.edu/

The Division of Biological Sciences at UCSD invites applications for Assistant, Associate, or Full Professor (tenure track or tenured) in stem cell biology, particularly from those working with human cells. All qualified applicants are encouraged to apply, including underrepresented minorities and women.

Area of scholarship is open. We seek candidates to complement our existing strengths in molecular, cell/developmental biology, and neuroscience, and who will foster research involving stem cells. Applicants must hold a Ph.D. degree or equivalent and should have outstanding records of research achievement; above the rank of Assistant Professor should have strong, extramurally supported research programs. Appointees are expected to participate fully in departmental affairs/teaching. Appointment level will be commensurate with qualifications and experience with salary based on UC pay scales. Applications will be reviewed beginning **March 15, 2005** and accepted until the positions are filled.

Mail hard copies of curriculum vitae, publication list, synopsis of professional goals, research/teaching interests, and three letters of reference (mailed directly from referees) to: **Stem Cell Biology Search Committee, c/o Heather Pratt - 0376-B, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0376.**

UCSD is an Equal Opportunity-Affirmative Action Employer with a strong institutional commitment to the achievement of diversity among its faculty and staff.

Max Planck Institute for Infection Biology, Berlin
The Department of Molecular Biology is seeking

Two Post-doctoral Fellows

for projects concerned with (i) the infection biology of Helicobacter pylori and the development of a human anti-H. pylori vaccine and (ii) the cell biology of bacterial infection (Salmonella, Neisseria and Chlamydia) and high-content RNAi and live microscopic analysis.

Candidates with a strong background in (i) microbiology/immunology and an interest in teamwork that spans from bench to bedside or, (ii) molecular and cellular biology of bacterial infections with an emphasis on bacterial phagocytosis and intracellular accommodation and processing, are encouraged to apply.

In addition we seek an experienced and committed

Technical microscopy expert (Biotechnologist)

interested in vastly independent, technically ambitious work in the context of our infection biological research including advanced confocal laser microscopy and live cell imaging, as well as electron microscopy.

All positions are initially available for two years with an option of further extension. Salary will be depending on your qualification. You can expect employee's benefits according to civil service.

The Max Planck Society is an equal opportunity employer and encourages applications from female candidates.

Please send applications (preferentially via e-mail) to:

Max Planck Institute for Infection Biology
Human Resources
Campus Charité Mitte
Schumannstraße 21/22
D-10117 Berlin / Germany
http://www.mpiib-berlin.mpg.de
job@mpiib-berlin.mpg.de

Medical University of South Carolina and the Hollings Cancer Center
Director Institute of Cancer Drug Development

This position is an exciting opportunity to develop a team of investigators who will explore new anticancer agents in the clinic, translating laboratory discoveries to the patient. The Institute will be housed in the new Hollings Cancer Center (HCC) laboratory space, the clinicians will be based in the Department of Medicine, and the basic researchers will have tenure track appointments in the basic science Departments in the School of Medicine.

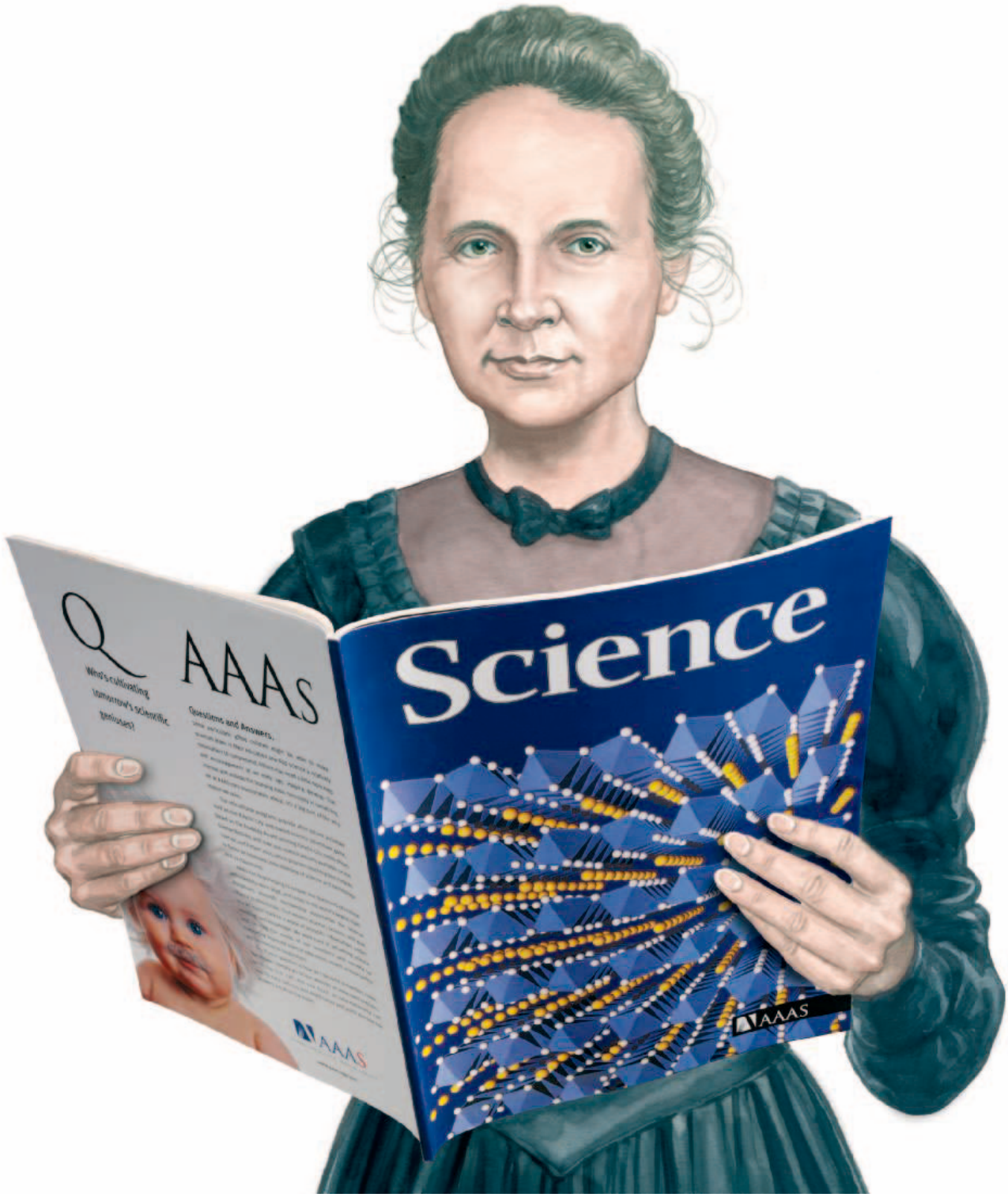
The successful applicant should have a significant track record in developing Phase I drug studies in the clinic, an independent clinical/basic research career with history of peer reviewed funding, and be a dynamic leader able to work within the Cancer Center matrix. The start-up package will include additional clinical recruitments and new laboratory space.

The HCC will occupy a new building housing radiation therapy, outpatient clinics, and laboratory investigators. The Center has programs in Experimental Therapeutics, Hormone-Dependent Malignancies, Tobacco-related Malignancies, Functional Genetics, Cancer Biology and Prevention and Control. The Hematology/Oncology Division occupies an inpatient floor, sees patients in multidisciplinary clinics, and has an active bone marrow transplant program. The Medical University of South Carolina is located within minutes of the beaches, outstanding fishing, the historic downtown and cultural venues, including the symphony, art, and history museums.

Interested candidates should send a copy of their curriculum vitae and the names of three references to: **Andrew S. Kraft, MD, Director Hollings Cancer Center, William H. Folk Chair in Experimental Oncology, 86 Jonathan Lucas Street, P.O. Box 250955, Charleston, S.C. 29425.** Or Fax to **843-792-9456** or e-mail to **Elisa Mundis** at **mundise@musc.edu**.

MUSC is an Equal Opportunity Employer, promoting workplace diversity.

MUSC
HOLLINGS CANCER CENTER



Marie Curie
1867–1934

ScienceCareers.org
We know science AAAS

EPA's Office of Research and Development (ORD) is seeking candidates to fill approximately nine federal, four-year post-doctoral research positions. Recent initiatives at ORD facilities have promoted the conduct of cross-cutting research across the different ORD Labs and Centers in the areas of human environmental exposure-effects and ecosystems. In the human health area, the overall mission for the cross-ORD post-docs will be to move forward more quickly the development and application of exposure, dose and health effects assessment methods or models. In the ecosystems research area, the cross-ORD post-docs will focus on advancing the spatial analyses methods and on their application to water quality, ecological forecasting problems, and linkages between economic drivers and landscape conditions. Applications will be accepted from December 13, 2004 through March 31, 2005.

The duty station and organization will be based on the particular disciplinary focus of the candidate and the overall requirements of the program. The positions will be in one of the following organizations: National Health & Environmental Effects Research Laboratory (NHEERL), National Center for Environmental Assessment (NCEA), National Exposure Research Laboratory (NERL), and the National Risk Management Research Laboratory (NRMRL). Duty stations associated with these organizations are: Research Triangle Park, NC, Cincinnati, OH, Las Vegas, NV, Washington, DC, Athens, GA, and Corvallis, OR.

These excepted service appointments offer a salary range of $48,947 - $76,261 and include a full benefits package. Applicants must be US citizens or permanent residents. For specific job information and application instructions, you may access the ORD Internet site at:
http://www.epa.gov/ord/htm/jobs_ord.htm

U.S. EPA is an Equal Opportunity Employer.

The Minestry of Food, Agriculture and Fisheries
The Danish Institute for Fisheries Research

Senior scientist

in fisheries management modelling

The Danish Institute for Fisheries Research (DIFRES), Department of Marine Fisheries, Copenhagen, Denmark, invites applications for a permanent position as a senior scientist in modelling fisheries management systems. We are looking for a key research profile to participate in developing methodologies for and practical development of models/software for fisheries management evaluations. The successful applicant is expected to strengthen the research profile of the fisheries section ant to significantly contribute to the development of the research area within the department.
Place of employment: Copenhagen, Denmark.

The full text of the announcement can be seen on www.dfu.min.dk. Application deadline: **1st April 2005, at 12.00 o'clock.**

Danish Institute for Fisheries Research (DIFRES) is a governmental research institution affiliated with the Ministry of Food, Agriculture and Fisheries. The institution carries out research, investigations and provides advice concerning sustainable and quality-focused exploitation of live marine and fresh water resources. Thus DIFRES deals with chain considerations from water to table. Danish Institute for Fisheries Research has approximately 280 employees located in Charlottenlund, Lyngby, Silkeborg and Hirtshals.

School of Medicine
New York University

New York University School of Medicine
Faculty Positions
in Medical and Molecular Parasitology

NYU School of Medicine is initiating a major expansion of the Department of Medical and Molecular Parasitology under the leadership of our new Chair, Dr. Karen Day. We are seeking outstanding candidates to fill **four** junior and senior level faculty positions.

The Department of Medical and Molecular Parasitology aims to achieve excellence in both **research** and **teaching** in the field of parasitology. We aim to discover and implement novel strategies to control parasitic diseases, such as malaria, that affect the health of millions worldwide. The Department is playing a key role in new global health initiatives, including a Masters in Global Public Health, at NYU.

Candidates working on **host/parasite systems** in any of the following research areas are encouraged to apply: **Genomics, Epidemiology, Genetics, Immunology, Biochemistry, Cell and Molecular Biology, Anopheline Biology**.

Ideal candidates will complement and expand the existing strengths in the Department. Candidates must have completed at least 5 years of postdoctoral training and developed an innovative research program in the above mentioned research areas. Competitive startup packages and newly renovated laboratory space are augmented by core facilities including insectaries, bioinformatics, confocal microscopy, animal facilities, flow cytometry. The Department is home to 13 faculty with active parasitology research programs and an NIH-funded graduate training program (**www.med.nyu.edu/parasitology**). See specific application instructions at **http://www.med.nyu.edu/parasitology/news/jobs.html**.

The New York University School of Medicine was founded in 1841 and is an Equal Opportunity, Affirmative Action Employer. Women and minority candidates are especially encouraged to apply.

nature immunology

Assistant Editor

Nature Immunology seeks an Assistant Editor to join their editorial team. Members of the editorial team evaluate manuscripts, oversee the peer review process, commission and edit Reviews, and write short pieces and editorials for the journal. The successful applicant will also attend meetings and conferences, visit laboratories and maintain contact with the international scientific community.

Candidates should have a broad interest in science, excellent communication skills, and the ability to work closely with others. Applicants will be required to have completed a Ph.D. or an M.D., and to have expertise in any of the areas covered in *Nature Immunology*. Special consideration will be given to candidates with expertise in the field of T cell signaling, but applications from candidates from other branches of immunology are also welcome.

Applicants should submit a Curriculum Vitae, a short News and Views article (of 500-1,000 words), and a cover letter explaining their interest in the position to: Human Resources Department, Nature Publishing Group, 345 Park Avenue South, New York, New York 10010, USA (fax: 212-696-9594; e-mail: admin@natureny.com. Applications should arrive as soon as possible, and no later than February 18. EOE.

nature publishing group npg

Rensselaer

Why not Change the World?

BIOLOGY FACULTY SEARCH

DEPARTMENT OF BIOLOGY

The Department of Biology at Rensselaer Polytechnic Institute seeks candidates in any area of basic biomedical research for tenure track faculty positions at all academic levels. We are particularly interested in candidates in the areas of genomics, proteomics, and bioinformatics as part of a campus-wide initiative in computational biology and bioinformatics.

Rensselaer has recently opened a 218,000 sq. ft. Center for Biotechnology and Interdisciplinary Studies with approximately 60 faculty laboratories and state of the art core facilities. Significant funding is available for startup packages.

Review of applications will begin now, but the search will continue until positions are filled. Please send a curriculum vitae, a statement of research interests up to three pages, and have a minimum of three letters of reference sent to:

Robert E. Palazzo, Director,
Center for Biotechnology and Interdisciplinary Studies,
Biology 1W14 SC, Rensselaer Polytechnic Institute,
110 8th Street, Troy, New York, 12180-3590

Contact Us at: Department of Biology, 1W14 Jonsson-Rowland Science Center, Rensselaer Polytechnic Institute, 110 Eighth Street, Troy, NY 12180-3590; Phone: (518) 276-6446.

Rensselaer Polytechnic Institute is an Equal Opportunity, Affirmative Action Employer. Members of underrepresented groups (including people of color, or with disabilities, and women) are strongly encouraged to apply.

CASE

CASE WESTERN RESERVE UNIVERSITY

Case Western Reserve University School of Medicine Endowed Chair in Inflammatory Bowel Disease Research

Applications are invited from qualified M.D., M.D.-Ph.D. or Ph.D. faculty to be appointed to the Victor and Ellen Cohn Chair for Inflammatory Bowel Disease at Case Western Reserve University/University Hospitals of Cleveland. The successful candidate will establish a cutting-edge research program to investigate the mechanisms and therapies of Crohn's disease and ulcerative colitis.

Applicants should have a strong academic track record commensurate with appointment as Professor or Associate Professor in the Department of Medicine (with tenure or tenure track) and an established research program with sustained extramural funding in areas relevant to inflammatory bowel disease, including inflammation, gastrointestinal pathophysiology, genetics and microbial pathogenesis, particularly if related to the enteric flora.

The successful candidate will join and expand an internationally renowned group of investigators with an established track record of basic science investigation in inflammatory bowel disease and mucosal immunity. In addition to the salary provided by the Chair's endowment, a generous package commensurate with the applicant's qualifications will be provided.

Interested applicants should forward a letter of interest, a curriculum vitae, the names and email addresses of three references, and a short summary of their research plans to the **Chairman of the Search Committee:**

Claudio Fiocchi, M.D, Division of Gastroenterology
Department of Medicine
University Hospitals of Cleveland/Case Western Reserve University
(BRB 425), 10900 Euclid Avenue, Cleveland, Ohio 44106-4952
Electronic format preferred to: jrp8@case.edu

Case Western Reserve University and University Hospitals of Cleveland are Affirmative Action/Equal Opportunity Employers and applications from women and minority investigators are particularly encouraged.

UNIVERSITY of VIRGINIA

DEAN

School of Engineering and Applied Science

The University of Virginia invites expressions of interest in, and nominations for, the position of Dean, School of Engineering and Applied Science.

The University of Virginia is a vigorous, modern institution, animated by the forward-looking spirit of its founder, Thomas Jefferson. In August 2004, U.S. News & World Report ranked the University of Virginia as the nation's #2 public university (tied with the University of Michigan), placing it 22nd overall among 248 public and private colleges and universities.

The University's 2020 Commission on Science and Technology recommended three focused, multidisciplinary initiatives, which were launched in 2002: a University-wide information initiative to build excellence in computer and information science and in engineering, an initiative in Quantum and Nanoscale Science and Engineering, and Morphogenesis and Regenerative Medicine.

The School of Engineering and Applied Science enrolls 2,645 students, has an annual budget of $70 million, including $44 million in externally funded research, a total full-time instructional/research faculty of 167, and offers BS, ME, MS, and Ph.D. degrees. As SEAS looks to its own future, four highly productive cluster areas have been identified for priority: bioengineering, computer and information science and engineering, nanotechnology, and societal and environmental systems.

The individual selected for this prominent appointment will be a distinguished scholar with impeccable academic credentials, will have a deep appreciation for the research enterprise of a great university, and will be recognized as an accomplished academic leader.

The Dean will have the breadth of view and experience needed to appreciate and celebrate the multitude of research-intensive disciplines and educational programs both within SEAS and the broader University. The Dean should have a vision for opportunities in engineering that will inspire faculty, students, and administrators. A high priority of this Deanship will be the ability to build new relationships with external stakeholders and friends of SEAS and to develop substantial new resources via capital fundraising initiatives.

The Dean of SEAS will be an effective leader who will understand and build upon U.Va.'s rich traditions and special resources as it continues as a premier institution. This person will take bold action and make decisions that reflect both intellect and foresight. This leader will have a clear sense of vision based on strongly held principles. The Dean will inspire others and instill pride in the School.

This position represents an extraordinary opportunity for a renowned scholar and academic leader to be the Dean of Engineering of one of our country's premier universities. All correspondence relating to the position of Dean of the School of Engineering and Applied Science should be directed in confidence to the University's executive recruitment consultant:

Dr. Randy Jayne and Karen Barrie
Heidrick & Struggles, Inc.
1750 Tysons Blvd., Suite 300
McLean, VA 22102
703-848-2500
kbarrie@heidrick.com

The University of Virginia is an equal opportunity/affirmative action employer committed to excellence through diversity.

POSITIONS OPEN

FACULTY POSITION
OPEN RANK
Department of Cell Biology and Anatomy and the Stanley S. Scott Cancer Center
Louisiana State University Health Sciences Center
New Orleans

A tenure-track faculty position at any rank is available in the Department of Cell Biology and Anatomy and the Stanley S. Scott Cancer Center at the Louisiana State University Health Sciences Center (LSUHSC) in New Orleans. The successful applicant is expected to pursue a successful career that includes research and teaching excellence. This is a joint recruitment involving the Department of Cell Biology and Anatomy and the Stanley S. Scott Cancer Center. The latter has partnered with the Tulane University Cancer Center to form the Louisiana Cancer Research Consortium (**website: http://lacrc.net**). Applicants must have a clear focus in cancer research, including active, or potential, funding by the National Cancer Institute, American Cancer Society, the Department of Defense, etc. Areas of particular interest include cancer genetics and DNA instability, molecular signaling (transcription factor, chromatin remodeling, cell-cell and cell matrix interaction, cell migration), and cell division, survival, and apoptosis. Teaching responsibilities will be in the area of cell and molecular biology for health professionals and graduate students.

To apply, please send curriculum vitae, statement of research interests, and the names of three references to: **Melissa Hebert, Business Manager, Department of Cell Biology and Anatomy, Louisiana State University Health Sciences Center, 1901 Perdido Street, Box P6-2, New Orleans, LA 70112-1393.**

LSUHSC is an Equal Opportunity/Affirmative Action Employer.

ASSISTANT/ASSOCIATE PROFESSOR
MICROBIOLOGY

The Department of Biological Sciences at San Jose State University (SJSU) invites applications for a tenure-track position in microbiology at the Assistant/Associate Professor level to begin in fall 2005. Applicants must possess a Ph.D. or equivalent degree and be a broadly educated microbiologist with a background in medical microbiology. Applicants should demonstrate a potential for excellence in teaching and an ability to establish a funded research program appropriate for an undergraduate-oriented university. Such a research program is expected to involve undergraduate and M.S. graduate students. The candidate will coordinate an allied health microbiology course and teach in other upper-division microbiology courses and graduate seminars. For consideration, send a letter of application, curriculum vitae, original university graduate transcripts, separate statements of teaching and research interests, and three letters of recommendation to: **Chair, Microbiology Search Committee, Department of Biological Sciences, San Jose State University, One Washington Square, San Jose, CA 95192-0100. Fax: 408-924-4840; telephone: 408-924-4900.** Review of applications will commence on February 18, 2005, and continue until the position is filled. Starting date for the position can be August 2005 or January 2006. See **website: http://www.sjsu.edu/depts/Biology** for more information. *SJSU is an Equal Opportunity/Affirmative Action Employer committed to the core values of inclusion, civility, and respect for each individual.*

ATMOSPHERIC SCIENTIST

Responsible for weather risk management analyses for insurance company. Ph.D. in physics, atmospheric sciences, or related field. Minimum one year experience including weather risk analysis, catastrophic risk modeling, and pricing/loss assessment for insurance industry. Mail curriculum vitae to: **P. Rademan, ACE, 1601 Chestnut Street, TLP 33K, Philadelphia, PA 19103.**

PIONEER
A DuPont Company

SENIOR RESEARCH ASSOCIATE

Pioneer Hi-Bred International, Inc is the world leader in the discovery, development, and delivery of elite crop genetics. We are looking for a Senior Research Associate for our Johnston, Iowa, location. This individual will provide antibody and enzyme-linked immunosorbent assay development support for crop genetics research and development. Responsibilities include monoclonal antibody screening, selection and evaluation, and developing and executing experimental strategies for assay development and validation. Bachelor's or Master's Degree in biological sciences plus three years or more related experience is required. Excellent communication skills are essential.

The Req ID for this position is RP539. For a complete job description and to apply, go to **website: http://www.pioneer.com/employment.** *Equal Opportunity Employer.*

BACTERIAL PATHOGENESIS
FACULTY POSITION
Department of Medical Microbiology and Immunology
The Medical College of Ohio

The Department of Medical Microbiology and Immunology invites applications for an **ASSISTANT/ASSOCIATE PROFESSOR,** tenure-track faculty position in the area of bacterial pathogenesis. We are particularly interested in candidates focused on immune evasion and emerging/select bacterial agents. Available facilities include a certified biosafety level three laboratory and microarray/proteomics/tissue culture core facilities. We seek highly motivated and collegial individuals with demonstrated research productivity, a doctoral degree, and relevant postdoctoral training. The appointee will be expected to maintain a strong, extramurally funded research program, participate in departmental teaching (particularly in the areas of bacterial genetics and pathogenesis), and interact productively with basic and clinical scientists focused on infectious diseases. Competitive salary, space, and startup funds will be provided. Applicants should send a brief statement of research interests, representative publications, curriculum vitae, and have three letters of recommendation forwarded to: **Professor Garry T. Cole, Medical College of Ohio, 3055 Arlington Avenue, Toledo, OH 43614-5806.** Applicant interviews will begin in March 2005 and will continue until the position is filled. Visit our **website: http://www.mco.edu/depts/micro/search.html.**

Affirmative Action/Equal Opportunity Employer.

GEOBIOLOGY PROGRAM
University of Southern California

The University of Southern California (USC) seeks applications for a tenure-track **ASSISTANT/ASSOCIATE PROFESSOR** position in geobiology. We seek applicants with skills and interests in bridging the earth and biological sciences with novel approaches and who will foster interdisciplinary research and teaching. Review of applications will begin in March 2005 and continue until the position is filled. Applications should include a complete resume, statement of research, and teaching experience. At least three references should be sent directly to: **Glen A. Smith (e-mail: glensmit@usc.edu)** and be addressed to the committee chair.

Dr. Kenneth H. Nealson
c/o Glen A. Smith
Geobiology Search Committee Chair
Allan Hancock Foundation
AHF 107G, MC 0371
University of Southern California
Los Angeles, CA 90089-0371

USC is an Affirmative Action/Equal Opportunity Employer.

EAST TENNESSEE STATE UNIVERSITY

ASSISTANT or **ASSOCIATE PROFESSOR** of Environmental Health. Two academic year tenure-track positions beginning fall 2005. Minimum qualifications include a graduate degree in environmental policy, environmental management, industrial hygiene, occupational health, environmental health, or environmental engineering. Ph.D. and postdoctoral experience preferred. Candidates that are all but dissertation will be considered. Must be able to teach courses in one of the following areas: policy and law, food sanitation and safety, solid and hazardous waste management, or occupational health and safety. Ability to teach other courses in the Department is desirable. The successful candidate(s) will demonstrate the ability to interface with a multidisciplinary faculty with a broad range of interests. Establishment of a funded research program in area of expertise that involves graduate (Master's) and undergraduate students is expected. Competitive startup funds are available. Applicants should send current curriculum vitae and names, addresses, e-mail addresses, telephone numbers of at least three references to: **Dr. Phillip Scheuerman, Chair, Department of Environmental Health, College of Public and Allied Health, East Tennessee State University, Box 70682, Johnson City, TN 37614.** Review of candidates will begin March 1, 2005, and continue until suitable candidates are found. *An Equal Opportunity/Affirmative Action Employer.*

CENTER DIRECTOR
Richard B. Russell Research Center (RRC)
U.S. Department of Agriculture

Agricultural Research Service (ARS). The RRC is located in Athens, Georgia, adjacent to the University of Georgia campus and includes offices, laboratories, greenhouses, storage facilities, and 27 acres of land. The Center is responsible for the development and dissemination of new technologies that address a wide range of problems that impact the U.S. poultry industry and food safety issues important to maintaining a safe food supply for the American consumer. The selectee will be responsible for providing leadership, coordination, and vision for the Center, and administration of its resources. Qualified candidates must have experience managing a scientific/professional staff; skill in managing multi-institutional programs; and knowledge of poultry and food safety research programs. *U.S. citizenship required.* Ph.D. preferred. ARS offers a comprehensive benefits package and negotiable recruitment incentives, including relocation assistance. Salary $82,438 to $126,064. For information concerning the position contact: **The Area Director's Office** at **telephone: 706-546-3311;** for application information contact: **Mrs. Genell Powers** at **telephone: 706-546-3029.** Applications must be postmarked by April 15, 2005. Visit our **website: http://www.ars.usda.gov/careers/Careers.htm.**

USDA/ARS is an Equal Opportunity Provider and Employer.

The Commodity Utilization Research Group, Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture (USDA), New Orleans, Louisiana, is seeking a permanent, **RESEARCH CHEMIST**, GS-1320-12/13. *U.S. citizenship is required.* Incumbent will participate in a research project to utilize recombinant DNA technology to engineer histidine acid phosphates for increased stability and specific activity to hydrolyze phytic acid found in animal feed. Incumbent will work with a team of scientists to develop an enhanced phytase for animal feed applications. A solid background in molecular modeling is required. Candidates must request a copy of vacancy announcement ARS-X5S-0083 by either calling **telephone: 301-504-1482** or by copying the announcement from **website: http://www.afm.ars.usda.gov/hrd/jobs/apply.htm** in order to respond/submit specific information outlined in the announcement. Applications must be postmarked by March 21, 2005. *USDA is an Equal Opportunity Provider and Employer.*

POSITIONS OPEN

DONALD DANFORTH PLANT SCIENCE CENTER

The Donald Danforth Plant Science Center announces openings for **PRINCIPAL INVESTIGATORS** at advanced levels. We seek highly motivated scientists with established research programs that complement those at the Danforth Center (**website: http://www.danforthcenter.org**). Of particular interest are candidates with interdisciplinary research that targets plant biology as it impacts human and animal nutrition/health, or production of bio-based materials/products. The Danforth Center is set in suburban St. Louis, Missouri, and offers an outstanding research environment with cutting-edge core facilities in proteomics, microscopy, and cell imaging, bioinformatics, plant cell/tissue culture, and plant growth. Opportunity for adjunct academic appointment at area universities. Salary will reflect level of experience and prior record. Applicants should submit curriculum vitae, selected reprints, and a description of future research to: **Ms. Billie Broeker, Human Resources Department, Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132. E-mail: bcbroeker@danforthcenter.org.**

Equal Opportunity/Affirmative Action Employer/Minorities/Females/Persons with Disabilities/Veterans.

SCIENTIFIC DIRECTOR
Center for Human Cell Therapy (CHCT)
CBR Institute for Biomedical Research
Children's Hospital, Boston
Harvard Medical School

The CBR Institute for Biomedical Research invites applications at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level for the position of Scientific Director of the Center for Human Cell Therapy. The CHCT is a newly NIH-funded facility at the CBR Institute for Biomedical Research in the Longwood Medical Campus. The mandate of the CHCT is to provide resources to all Harvard Medical School faculty to facilitate bench-to-bedside development of cellular therapies–including therapies based on adult or embryonic stem cells–for the treatment of damaged or diseased tissues. The Scientific Director of the CHCT will have close interaction with ongoing related research programs at the Harvard affiliated hospitals and the Harvard Stem Cell Institute. The incumbent of this position will be provided with startup funds and space to direct his/her own extramurally funded research program in areas related to stem cell or immunotherapy, such as stem/somatic cell biology, cell trafficking, or transplantation biology. The incumbent will also provide oversight of the Translational Cell Therapy Laboratory, which provides direction to principal investigators engaged in translational research projects involving human cells. An M.D. and/or Ph.D. with evidence of outstanding investigation and a record of collaborative interaction are essential. Prior experience overseeing a clinically related resource, i.e., the development of human cells for therapy, is desirable but not required. To apply, submit curriculum vitae, statement of research interests/plans, and names of three references via mail to: **Leslie E. Silberstein, M.D., Director, Joint Program in Transfusion Medicine, Children's Hospital Boston, Karp Family Research Building, 1 Blackfan Circle, 10th Floor, Room 10217, Boston, MA 02115.**

Affirmative Action/Equal Opportunity Employer.

POSTDOCTORAL POSITION available in immunology, protein biochemistry, microbiology, or molecular biology to study novel innate immunity genes and their functions in transgenic mice, humans, and mammalian cells in vitro. Send curriculum vitae and names of references to: **Dr. Roman Dziarski, Indiana University School of Medicine, 3400 Broadway, Gary, IN 46408, U.S.A. Website: http://shaw.medlib.iupui.edu/nwcme/smpledsk.html. E-mail: rdziar@iun.edu.**

POSITIONS OPEN

DIRECTOR
Program in Stem Cell Biology
Yale University
School of Medicine

Yale School of Medicine seeks an internationally recognized academic leader to head a new Program in Stem Cell Biology. Qualified candidates must have strong research credentials and proven leadership ability. This interdepartmental program will provide a scientific focus for already strong efforts in developmental biology, neurobiology, vascular biology, hematology, immunology, transplantation, biomedical engineering, and translational research. We encourage applications from individuals with interests in the fundamental aspects of mammalian stem cell biology, but candidates with other relevant interests will also be considered. Qualifications include M.D., Ph.D., or M.D./Ph.D. degree. Candidates are expected to meet the requirements for appointment to the senior faculty ranks. Interested candidates should electronically send curriculum vitae, brief statement of programmatic goals, and names of references to **e-mail: geraldine.emerling@yale.edu.**

Diane Krause, M.D., Ph.D.
Chair, Search Committee
c/o Gerri Emerling, Coordinator
Yale School of Medicine
333 Cedar Street, P.O. Box 208000
New Haven, CT 06520-8000

Yale University is an Equal Opportunity/Affirmative Action Employer. Qualified women and minority group members are encouraged to apply.

CHIEF, PULMONARY UNIT. The Department of Medicine of the University of Rochester School of Medicine and Dentistry is recruiting for the position of Chief, Pulmonary Unit. We are looking for candidates with a strong record of independent research in pulmonary biology, administrative leadership, and commitment to patient care and education. Preference will be given to individuals with experience in the areas of inflammation and immunology, pulmonary vascular disease, cancer, and genetics. The successful candidate will be expected to foster the growth of innovative high quality research and patient care programs in the Unit. Resources, including additional faculty positions, laboratory space in a newly constructed research building, office space, and startup funds (sufficient for four faculty recruits) are available to strengthen the highly successful Pulmonary Unit. The successful candidate (M.D. or M.D., Ph.D.) should have the academic qualifications for a tenure-track appointment at the level of **ASSOCIATE** or **FULL PROFESSOR.** The University of Rochester has undertaken a 10-year, $400 million strategic plan to expand the Medical Center's research programs. This recruitment is an integral component of the strategic plan and a high priority. Interested applicants should contact: **Mark Taubman, M.D., Chief of Cardiology, 601 Elmwood Avenue, Box 679, Rochester, NY 14642. E-mail: mark_taubman@urmc.rochester.edu.** *The University of Rochester is an Equal Opportunity Employer and applications from women and members of minority groups are encouraged.*

Bacterial Pathogenesis – Two **POSTDOCTORAL POSITIONS** are available: (1) to study the genetic *Francisella tularensis.* The research will focus on determining which genes from *F. tularensis* are necessary for survival within macrophages and infection of mice. This position requires select agent clearance; (2) to study virulence regulation through quorum sensing in enterohemorrhagic E. coli. Candidates with a Ph.D. and a strong background in molecular biology and prokaryotic gene regulation are highly desired. Please provide curriculum vitae and the names and addresses of three references to: **Dr. Vanessa Sperandio, Department of Microbiology, U.T. Southwestern Medical Center, 6000 Harry Hines Boulevard, Dallas, TX 75390-9048. Fax: 214-648-5905; e-mail: vanessa.sperandio@utsouthwestern.edu.** *U.T. Southwestern Medical Center is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

RTI INTERNATIONAL

TOXICOLOGIST

The Science and Engineering Group of RTI International (Research Triangle Institute) is seeking a candidate with a Ph.D. in toxicology to conduct research in proteomics as applied to toxicological research problems. The individual will be responsible for leading research projects in the Proteomics Research Group, working with existing and new clients, in both the commercial and government sector, writing winning proposals, and collaborating with a highly interdisciplinary team of scientists in biochemistry, analytical chemistry, proteomics, and bioinformatics. Strong communication and teaming skills are necessary.

The applicant should have a Ph.D. degree in toxicology and at least eight or more years of experience in the use of proteomic technologies as applied to toxicology directed studies. The candidate should possess knowledge of routine and research applications of modern proteomics technologies, including two-dimensional gel electrophoresis and mass spectrometry. Experience shall include project management, experimental design, data coordination, and informatics in proteomic studies. The candidate should also have experience in managing, mentoring, and directing the work of junior personnel. A strong record in publishing articles in the peer-reviewed literature and experience in preparing proposals to external funding agencies is mandatory.

Please apply online at **website: http://www.rti.org/careers.** Requisition No. CS11123.

We are proud to be an Equal Employment Opportunity/Affirmative Action Employer. Minorities/Females/Persons with Disabilities/Veterans.

POPULATION BIOLOGIST. The Biology Department of Franklin & Marshall College invites applications for a one-year **VISITING ASSISTANT PROFESSOR** position in population biology pending administrative approval, starting July 2005. Candidates should have the Ph.D.; demonstrated strengths in teaching and research in both field and laboratory settings; broad interests in population biology, evolution, and ecology; and strong quantitative abilities. Teaching responsibilities will include lectures and laboratories in an evolution-centered introductory course that includes Mendelian genetics and ecology, and an upper-level elective in the candidate's area of expertise. Franklin & Marshall is a small (enrollment 1,800), highly selective coeducational liberal arts college with a tradition of excellence in science and student research. Applicants should arrange to have letters sent from three referees, and should submit curriculum vitae, plans for actively engaging undergraduates through teaching and research, and undergraduate and graduate transcripts. Priority will be given to completed applications received by March 18, 2004. Send applications to: **Dr. Mark Olson, Department of Biology, Franklin & Marshall College, P.O. Box 3003, Lancaster, PA 17604. Telephone: 717-291-4118; fax: 717-358-4548; e-mail: cindy.mcintyre@fandm.edu; website: http://www.fandm.edu/biology.xml.** *The College is committed to cultural pluralism through the hiring of minorities and women. Equal Opportunity Employer/Affirmative Action.*

One or two NIH-funded **POSTDOCTORAL POSITIONS** available to study: (1) the role of hepatocyte nuclear factor-4$f\tilde{N}$ in redox regulation of hepatocyte inductible nitric oxide synthase gene transcription, (2) the role of nitric oxide in upregulation of osteopontin gene transcription, and/or (3) the effect of redox on chromatin remodeling. M.D.-Ph.D. or Ph.D. candidates with a strong background in the molecular biology of transcription, protein-protein interactions, or protein-DNA interactions are encouraged to apply. Please send curriculum vitae to: **Paul C. Kuo, M.D., Duke University Department of Surgery, Box 3522, 110 Bell Building, Durham, NC 27710. E-mail: kuo00004@mc.duke.edu.**

GRANTS

THE SONTAG FOUNDATION

Distinguished Scientist Award

Three-year awards totaling up to $500,000

Through Distinguished Scientist Awards, The Sontag Foundation seeks to fund innovative brain cancer research while providing support and recognition to help advance the careers of outstanding brain tumor researchers who have recently attained an initial faculty appointment. These early career scientists must show exceptional promise of impacting brain cancer research through projects exhibiting the potential to lead to increased rates of survival and/or improved functional recovery for individuals with brain tumors.

Application and eligibility guidelines:
www.sontagfoundation.com

Application Deadline: March 22, 2005
Start of Award: On or about October 1, 2005

Eight outstanding researchers are currently being funded through The Sontag Foundation Distinguished Scientist Awards. Three additional awards are anticipated in 2005.

For further information please contact: The Sontag Foundation
822 A1A North, Suite 300 • Ponte Vedra Beach, FL 32082
904.273.8755 • email: kverble@sontagfoundation.com

RESEARCH OPPORTUNITIES

VIRGINIA BIOINFORMATICS INSTITUTE — VIRGINIA TECH — VBI

Through joint research collaboration between Virginia Bioinformatics Institute (VBI) at Virginia Polytechnic Institute and State University (informally known as Virginia Tech) and University of Maryland, a **Rickettsiae/Coxiella Bioinformatician** is sought to collaborate with a team of scientists. Will work with rickettsial pathogens and hands-on rickettsial molecular biology, bioinformatics, etc. This 2-year appointment will be housed initially at the University of Maryland, College Park, MD and later at VBI at Virginia Tech, Blacksburg, VA. PhD in medical or biological sciences or related field, or equivalent experience with significant experience in infectious diseases and knowledge or working experience with large-scale data sets, such as genomes, transcriptomes, proteomes, etc., required. Posting number 041864

Functional Genomics Data Analysis Postdoctoral Associate to research and develop an informatics system to support functional genomics data and combine it with analytical chemistry data. PhD in biological sciences with knowledge of statistics, computing or computer science with experience of research applied to biological problems, or equivalent. Knowledge in at least two of the following areas: functional genomics, biochemistry or molecular biology, statistics and computer science. Research in microarray, proteomics, or metabolite profiling required. Posting number 040846.

Metabolomics and Senior Metabolomics Specialists will collaborate with a group of scientists to develop and validate new approaches and methods for high throughput analyses of complex biological matrices using LC-MS and CE-MS technologies. Ph.D. in Chemistry, Biochemistry or related field, or equivalent experience; demonstrative laboratory experience relevant in organic or inorganic chemistry; and experience with one or more of the following technologies required: HPLC and capillary LC, capillary electrophoresis, LC-MS, or CE-MS. Posting numbers 041779 and 041781.

For More Information:

To Apply, visit www.jobs.vt.edu and search by posting number.
To learn more about VBI and more career opportunities, please visit us at www.vbi.vt.edu.

Virginia Tech

To learn more about the Interdisciplinary PhD program in Genetics, Bioinformatics, and Computational Biology, visit www.grads.vt.edu/gbcb/overview.htm.

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Europe and International
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THE INTERNATIONAL VACCINE INSTITUTE

POSITION ANNOUNCEMENT

Institute Deputy Director for Laboratory Sciences

The International Vaccine Institute (http://www.ivi.org) is seeking an **Institute Deputy Director** to oversee the operations and continue the development of the Institute's Division of Laboratory Sciences, which will ultimately occupy approximately 90,000 sq feet of floor space in the Institute's new headquarters facility.

The International Vaccine Institute is non-profit and focuses on research and development of new and improved vaccines for use in developing countries. The Institute's headquarters are in Seoul, Korea.

The Laboratory Division currently has 7 principal investigator scientists working in laboratories devoted to mucosal immunology, cellular immunology, humoral immunology, bacterial genetics and bioinformatics, and vaccine development and process research. The Division conducts both basic and applied research. Current laboratory work complements and takes advantage of the Institute's ongoing epidemiologic and clinical research programs, which are being undertaken in 21 countries in Asia, Africa, and Latin America. The Division's operating and research budget is supported by both grants and core funding.

While it is expected that the incumbent will possess a broad view of the entire field of vaccine research, he or she should also be a recognized leader in a field relevant to the Institute's laboratory research programs. It is expected that the incumbent will conduct a program of independently funded research in his or her areas of interest, while maintaining overall responsibility for the Institute's laboratory research programs. The incumbent should also possess clear strengths in program-building, resource mobilization, and staff development. Minimum qualifications include a doctorate degree in a relevant discipline, and significant experience in leading multi-person laboratory efforts in a research environment.

Salary will be internationally competitive. The Institute provides attractive fringe benefits including a housing allowance, home leave, and income tax reimbursement.

The International Vaccine Institute is an independent international organization established under the Vienna Convention of 1969. 35 countries and the World Health Organization are signatories to the Institute's Charter. The Institute is governed by an international Board of Trustees, the majority of whom are elected based on their personal achievements and positions in the vaccine field.

Letters of interest along with a current *curriculum vitae* should be addressed to: **Mr. Hong-ki Jong, Senior Administrative Officer, International Vaccine Institute, Kwanak PO Box 14, Seoul, Korea. Tel: 82-2-872-2801 Fax: 82-2-872-2803 Email: hjong@ivi.int** from whom further particulars can be obtained. Absolute confidentiality will be respected.

POSITIONS OPEN

TENURE-TRACK FACULTY POSITION
Immunoparasitology

The George Washington University Medical Center is expanding its program in the Department of Microbiology and Tropical Medicine (**website: http://www.gwumc.edu/microbiology**). We are now seeking applicants for a tenure-track position at the **ASSISTANT PROFESSOR** level whose research focus is within the area of tropical and infectious diseases, with special interest in immunology and vaccine development. The successful candidate will be expected to establish an independent, fully funded research program that will complement the research activities of existing faculty in infectious diseases, vaccine biology, and immunology. We are particularly interested in individuals studying host-parasite interactions and their application to vaccine development and testing in animal models.

Required: A Ph.D. in microbiology, immunology, or infectious diseases with postdoctoral training is a prerequisite. Preferred qualifications: Applicants holding a D.V.M. or equivalent degree and previous experience in process development, vaccine formulation, potency, and Quality Assurance/Quality Control (QA/QC) are especially encouraged to apply. The successful candidate will have an ongoing sponsored research program, and some teaching experience.

Review of applications will begin on March 20, 2005, and will continue until the position is filled. Submit curriculum vitae, a one- to two-page statement outlining research and teaching interests, and the names of three references to:

Peter Hotez, M.D., Ph.D.
Department Microbiology and Tropical Medicine
The George Washington University
Ross Hall, Room 736
2300 Eye Street, N.W.
Washington, DC 20037
Telephone: 202-994-3532
Fax: 202-994-2913
E-mail: mtmpjh@gwumc.edu

The George Washington University is an Equal Opportunity/Affirmative Action Employer.

POSTDOCTORAL POSITION. Kidney Biology and Pathobiology, Case School of Medicine (**website: http://www.metrohealthresearch.org/renal.html**). Unexpected opening on NIH institutional training grant beginning July 2005. Training faculty use cell, molecular biology, and genetic techniques to understand normal kidney function and mechanisms of kidney disease. Research interests include identification of nephropathy susceptibility genes, expression profiling at an RNA and protein levels, analysis of transcriptional regulation, regulation and function of novel kidney ion channels and transporters, control of protein trafficking, podocyte biology, extracellular matrix receptors and signaling, kidney development, effects of mechanical force on cells, and mucosal immunity. M.D. or Ph.D. candidates are welcome. Experience in molecular biology, cell biology, biochemistry, or electrophysiology is desirable. Must be permanent resident. Send letter of application, curriculum vitae, and letters of recommendation, c/o **e-mail: nkessler@metrohealth.org.**

POSTDOCTORAL FELLOW POSITION

A Postdoctoral position is available to investigate flagellin glycosylation and the role of flagella in virulence of *Campylobacter jejuni*. The position is located in the laboratory of **Dr. Patricia Guerry,** Enteric Diseases Department of the Naval Medical Research Center in Silver Spring, Maryland, a suburb of Washington, D.C. Candidates must have a Ph.D. degree in microbiology, molecular genetics, chemistry, or related discipline. Please send applications, including detailed curriculum vitae and contact information for three references to **website: http://www.hjf.org.** Attn: Job Req. 200973. *Affirmative Action/Equal Employment Opportunity.*

POSITIONS OPEN

UNDERGRADUATE SUMMER RESEARCH OPPORTUNITIES

During the 10-week (May 31–August 5, 2005), NIH-supported program, students will conduct original research under the supervision of faculty members of the Department of Pharmacology. Students will work on problems at the frontier of biomedical receptor and signaling research using state-of-the-art techniques. In addition, students will participate in a foundations of signal transduction course and workshops. At the end of the summer, the students will present their work at a culminating colloquium.

Application deadline: March 1, 2005; salary: $5,000.

Eligibility: chemistry, math, physics, computer science, engineering majors who have completed their sophomore or junior years. *U.S. citizen or permanent resident status.*

For application information: **website: http://www.pharmacology.us/; Department of Pharmacology, University of Pittsburgh School of Medicine, W1340 Biomedical Science Tower, Pittsburgh, PA 15261. Telephone: 412-648-9321; fax: 412-648-1945; e-mail: summer@server.pharm.pitt.edu.**

MICROSCOPY AND IMAGING CORE FACILITY MANAGER

The Neurobiology Program of Children's Hospital Boston, Harvard Medical School, seeks a System Manager for the Imaging Core Facility of the NIH-funded Mental Retardation Research Center. The Core serves as a resource for single and multi-photon laser scanning confocal microscopy, digital microscopy, and advanced image analysis to investigators throughout Children's Hospital and Harvard Medical School. Applicants should be knowledgeable in the use and maintenance of confocal and standard microscopes, and image analysis software programs. Responsibilities will include basic Core administration, assisting investigators with use of microscopy equipment, and the design of new imaging techniques, and maintaining the Core instruments. Preference will be given to candidates with training or experience in multi-label intracellular localization, fluorescence recovery after photobleaching, fluorescence resonance energy transfer, and tissue slice, live cell, and whole mount imaging. Good communication skills are essential.

Minimum requirements include a Bachelor's or Master's degree in a related field with microscopy experience. Send resume and two letters of reference to:

Scott L. Pomeroy, M.D., Ph.D.
Imaging Core Director
Mental Retardation Research Center
Neurobiology Program
Enders 260
Children's Hospital Boston
300 Longwood Avenue
Boston, MA 02115
E-mail: scott.pomeroy@childrens.harvard.edu

POSTDOCTORAL POSITIONS

Two NIH-funded positions are available in the area of flavoprotein enzymology in the laboratory of **Dr. Marilyn Jorns** at Drexel University College of Medicine, Department of Biochemistry and Molecular Biology, Philadelphia, Pennsylvania. These positions involve mechanistic studies on bacterial sarcosine oxidases and nikD, an enzyme important in the biosynthesis of nikkomycin antibiotics (**website: http://www.drexel.edu/med/biochemistry/faculty/resume_jorns.htm**). A general background in biochemistry with experience in mutagenesis/kinetics and molecular biology is desirable. Please forward curriculum vitae and the names/contact information of three references to **e-mail: marilyn.jorns@drexelmed.edu.**

POSITIONS OPEN

The Albert Einstein College of Medicine seeks a tenure-track **ASSISTANT/ASSOCIATE PROFESSOR** in bioinformatics, who will serve as a Director of the Bioinformatics Shared Resource (BSR). The BSR is a data management/data mining facility, serving the research needs of the entire medical school faculty. The successful applicant will oversee a group of bioinformatics programmers, and actively collaborate with basic and clinical science researchers to provide data management and bioinformatics analysis support. The position is highly collaborative in nature and provides an opportunity to establish an independent research program. The BSR collaborates closely with statisticians, other bioinformatics research faculty, and various AECOM shared resource facilities in proteomics, genetics, and clinical research. Screening of applications will be continuous. Applications received by March 1, 2005, are assured full consideration.

Ref: Assistant/Associate Professor, Director of Bioinformatics.

Method of applying: Please send detailed curriculum vitae with bibliography, and contact details (telephone number, e-mail address) for at least three references to **e-mail: omendoza@aecom.yu.edu.**

Subject: Director of BSR; ATTN: Chair, Faculty Search Committee for BSR

Equal Opportunity Employer.

FACULTY POSITION IMMUNOLOGY/PATHOLOGY

The University of Utah, Department of Pathology, seeks qualified candidates in immunology for a tenure-track position at the **ASSISTANT/ASSOCIATE PROFESSOR** level, with emphasis on host responses to bacterial, viral, or parasitic infections.

Candidate should have a Ph.D. and/or M.D., and a record of scholarly, laboratory-based achievements with strong potential for independent, extramurally funded research.

Curriculum vitae and brief statement of current and future research should be submitted by April 1, 2005. Please include the names of three individuals who can provide recommendation letters.

c/o Janis Weis, Ph.D.
Department of Pathology
University of Utah
School of Medicine
30 N 1900 East Room 5C124
Salt Lake City, UT 84132

Website: http://www.path.utah.edu/cbi/index.htm.

The University of Utah is an Equal Opportunity/Affirmative Action Employer.

ASSOCIATE RESEARCH SCIENTIST
Columbia University Institute for Cancer Genetics

Associate Research Scientist positions are available to study the molecular pathogenesis of various cancers, including breast, lymphoid, prostate, and brain. Ph.D. or M.D. and extensive related research experience required.

Please send statement of research interests, curriculum vitae, availability, and names of references to: **Dr. Linda Lowenstein, Institute for Cancer Genetics, Columbia University, 1150 St. Nicholas Avenue, New York, NY 10032. Fax: 212-851-5256** or **e-mail: ls35@columbia.edu.**

Columbia University is an Equal Opportunity/Affirmative Action Employer.

POSTDOCTORAL POSITION
University of California, San Francisco

Available to study neuronal circuitry underlying behavior in zebrafish. Candidates should have a strong background in molecular biology, microscopic imaging, and neurobiology. Please send curriculum vitae and names of three references to: **Dr. Su Guo, Department of Biopharm. Sciences, University of California, San Francisco, CA 94143-2911. E-mail: suguo@itsa.ucsf.edu.**

Cold Spring Harbor Laboratory 2005 Meetings

Protein Phosphorylation & Cell Signaling

May 18 - 22, 2005
Abstracts Due February 23

Organizers

Sara Courtneidge, Van Andel Research Institute
Benjamin Neel, Beth Israel Deaconess Medical Center
Nick Tonks, Cold Spring Harbor Laboratory

Keynote Addresses

Craig Thompson, University of Pennsylvania
Martine Roussel, St. Jude Children's Research Hospital

Topics include

Kinase/Phosphatase Pathways in Metabolism
Cell Cycle
Kinase/Phosphatase Abnormalities in Human Disease
Immune Cell Signaling
Cytoskeleton and Cell Adhesion Signaling
Signaling by Receptor Kinases & Phosphatases
Neuronal Signaling

Session Chairs

Robert Abraham, Burnham Institute
Dafna Bar-Sagi, Stony Brook University
Doreen Cantrell, University of Dundee
Jack Dixon, UC San Diego
Kathy Gould, HHMI / Vanderbilt University
Bill Muller, McGill University

Other 2005 Meetings

Target Definition & Vector Design for Molecular Medicine
March 3 - 6

Imaging Neurons & Neural Activity
March 10 - 13

Systems Biology: Global Regulation of Gene Expression
March 17 - 20

Learning & Memory
April 20 - 24

The Ubiquitin Family
April 27 - May 1

Telomeres & Telomerase
May 4 - 8

The Biology of Genomes
May 11 - 15

Retroviruses
May 24 - 29

70th Symposium: Molecular Approaches to Controlling Cancer
June 1 - 6

Yeast Cell Biology
August 16 - 21

Eukaryotic mRNA Processing
August 24 - 28

Mechanisms of Eukaryotic Transcription
August 31 - September 4

Eukaryotic DNA Replication
September 7 - 11

Microbial Pathogenesis & Host Response
September 14 - 18

Programmed Cell Death
September 21 - 25

RNAi
September 28 - October 2

Neurobiology of *Drosophila*
October 5 - 9

Genome Informatics
October 26 - 30

Molecular Approaches to Vaccine Design
December 1 - 4

Rat Genomics & Models
December 8 - 11

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POSITIONS OPEN

COMPUTATIONAL SCIENTISTS
Gene Expression Biostatistics and Data Integration

Computational scientists will be responsible for analysis of microarray data, integration of the results with other proprietary and publicly available data, design and implementation of high throughput systems for genomic and metabolic pathway discovery, and development of data mining interfaces. The candidates should be highly motivated, have a Ph.D. or M.Sc. in statistics, computer science, physics, or biology, a solid background in statistics and algorithm development, extensive experience of experimental data analysis, and proficiency in programming. Experience with commercial data analysis software Genespring in desirable. They also should have at least four years of software development experience in UNIX or Microsoft environments, with proven experience in Java, HTML, JSP, and web interface development. The individuals must be proficient in SQL, PL/SQL, and UNIX shell scripting, be familiar with database design, and possess excellent communication skills. Experience in the following areas is a plus: Visual Basic, XML, and PERL, J2EE and application frameworks (Spring, Webwork, etc.). Successful candidates will be able to work closely with staff scientists on different biological projects. Please refer to Job Code Bi-0105.

Ceres, Inc. has moved to a new state-of-the-art research facility in the hills of Thousand Oaks. We offer competitive salaries and excellent benefits including equity participation and a 401K. Highly motivated individuals should send applications, including current curriculum vitae, summary of research experience, and names and addresses of three references to: **Human Resources Manager, Ceres, Inc., 1535 Rancho Conejo Boulevard, Thousand Oaks, CA 91320;** or **e-mail** to: **ceres-hr@ceres-inc.com.** The positions are available immediately.

Department of Health and Human Services
National Institutes of Health
National Cancer Institute

Two **POSTDOCTORAL POSITIONS** are available with the Laboratory of Protein Dynamics and Signaling, Center for Cancer Research (CCR), National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health and Human Services (DHHS) located in Frederick, Maryland, to: (1) study the role of the ubiquitin proteasome system in Parkinson's Disease and (2) study structure/function relations of ubiquitin ligases and participate in the development of small molecules that modulate the activity of ubiquitin ligases that are implicated in cancer. Background in cancer cell biology, biochemistry, or neurobiology is highly desirable. Candidates should have a recent M.D. and/or Ph.D. and less than five years of postdoctoral experience.

Salary is commensurate with research experience. Please apply electronically by sending letter of interest, curriculum vitae, and names of three references to: **Allan M. Weissman** at **e-mail: amw@nih.gov.**

DHHS and NIH are Equal Opportunity Employers.

POSTDOCTORAL POSITIONS are available to examine the structure and function of porins of *Mycobacterium tuberculosis.* The unique outer membrane of Mtb is an efficient permeability barrier that is the major determinant of the intrinsic resistance of mycobacteria to most antibiotics. The ultimate goal of this NIH-funded project is to exploit this knowledge for the design of more effective TB drugs. For further information see: *Mol. Microbiol.* **49:**1167, 2003; *Science* **303:**1189, 2004.

Supplementation to NIH salary levels is available for qualified candidates. The new laboratory, the Department of Microbiology, and the University of Alabama at Birmingham provide an outstanding research environment. The establishment of independent projects is encouraged. Please send curriculum vitae and three letters of recommendation to: **Michael Niederweis, Ph.D., e-mail: mnieder@uab.edu.** *UAB is an Equal Opportunity Institution.*

POSITIONS OPEN

POSTDOCTORAL AND CLINICAL FELLOWSHIPS
at the
National Institutes of Health
U.S. Department of Health and Human Services

Website: http://www.training.nih.gov

NIH is dedicated to building a diverse community in its training and employment programs.

FACULTY POSITION
The University of Chicago
Gwen Knapp Center for Lupus and Immunology Research

The Gwen Knapp Center for Lupus and Immunology Research at the University of Chicago is seeking a tenured position in immunology. This appointment is part of an exciting and renewed expansion of the Center and the Department of Pathology.

Successful applicants should hold an M.D., Ph.D., or M.D./Ph.D. and have an outstanding record of published research and sustained peer-reviewed funding. The academic rank will be commensurate with qualifications and will be in the Department of Pathology. Applicants must have a strong background in lupus research. A commitment to education (residency training and/or graduate or medical student training) is essential. The Knapp Center offers excellent laboratory facilities and access to outstanding core facilities at the University of Chicago including flow cytometry, confocal/immunocelectron microscopy, DNA and peptide synthesis, microarray facility, and immunohistochemistry. A highly competitive startup package is available.

Applicants should submit by, March 1, 2005, their curriculum vitae, a short research summary with plans for further research, and three letters of recommendation to: **Martin Weigert, Ph.D., Director, Gwen Knapp Center for Lupus and Immunology Research, The University of Chicago, 924 East 57th, R413A, Chicago, IL 60637. E-mail: mweigert@bsd.uchicago.edu.**

The University of Chicago is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply.

BOSTON HARBOR ISLANDS POSTDOCTORAL FELLOWSHIP

The Department of Entomology in the Museum of Comparative Zoology (MCZ) invites applications for the Boston Harbor Islands Postdoctoral Fellowship, located in the existing biodiversity and island biogeography program in the MCZ. The incumbent will work with MCZ and National Park Service (NPS) personnel to develop an All Taxa Biotic Inventory (ATBI) with emphasis on insects and other invertebrates located on the Boston Harbor Islands national park area. Additional expectations include participation in educational outreach; for example, assisting in the development of an online database of the ATBI ongoing results and linking them to **websites: http://insects.oeb.harvard.edu** and **http://www.bostonislands.org,** and in the development of a public exhibit for the Harvard Museum of Natural History using this data obtained. The Fellowship is available for three years, with a desired start date of May 1, 2005, and possibility of renewal for two additional years. Please send a cover letter with a statement of relevant experience and intent, curriculum vitae, representative publications, and arrange to have three letters of reference sent to:

Committee for Boston Harbor
Postdoctoral Fellowship
Department of Entomology
Museum of Comparative Zoology
26 Oxford Street
Cambridge, MA 02138

Review of applications will begin on March 15, 2005. *Harvard University is an Equal Opportunity Employer.*

POSITIONS OPEN

POSTDOCTORAL RESEARCH FELLOW

The Division of Rheumatology at The University of Texas Southwestern Medical Center at Dallas is seeking a Postdoctoral Research Fellow with experience in the area of immunology and inflammation. The successful candidate must have a Ph.D. or equivalent in immunology, nephrology, or related disciplines and is expected to engage in externally sponsored research programs in lymphocyte and dendritic cell responses to oxidative stress and autoimmune inflammation, present data, and to publish in peer-reviewed journals. Interested candidates should submit a letter of application designating current and long-term research interest, curriculum vitae, and the names and addresses of three references to:

David R. Karp, M.D., Ph.D.
Associate Professor of Internal Medicine
Chief, Rheumatic Diseases Division
UT Southwestern Medical Center
5323 Harry Hines Boulevard
Dallas, TX 75390-8884
E-mail: david.karp@utsouthwestern.edu

UT Southwestern Medical Center is an Equal Opportunity/Affirmative Action Employer.

ASSOCIATE RESEARCH SCIENTIST and/or **POSTDOCTORAL RESEARCH SCIENTIST** position available immediately in the Department of Pharmacology at Columbia University. This is an opportunity to join an interdisciplinary team investigating the molecular basis of inherited mutation-induced rhythm disturbances in the heart. Candidates must have either Ph.D. or M.D. degrees and must have experience appropriate for the positions. Expertise in protein chemistry and/or biochemistry of membrane and signaling molecule proteins is required. Send curriculum vitae to: **Dr. Robert S. Kass, Ph.D., Department of Pharmacology, Columbia University College of Physicians and Surgeons, 630 West 168th Street, New York, NY 10032.**

We are an Equal Opportunity/Affirmative Action Institution.

POSTDOCTORAL FELLOWSHIP position is available at Northwestern University, to work in projects on the mechanisms of interferon and retinoic acid signaling in malignant cells. Previous experience in molecular biology/biochemistry preferred. Applicants should submit their curriculum vitae to: **Leonidas C. Platanias, M.D., Ph.D., Professor of Medicine, Deputy Director, Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Feinberg School of Medicine, Olson 8250, 303 East Chicago Avenue, Chicago, IL 60611. E-mail: l-platanias@northwestern.edu.**

The Plant Science Research Center at the University of Toledo has two openings for **POSTDOCTORAL RESEARCH ASSOCIATES,** three openings for **RESEARCH LABORATORY TECHNICIANS,** and one opening for a **GREENHOUSE TECHNICIAN.** Please visit **website: http://psrc.utoledo.edu** for position descriptions and further information. Review of applications will begin from February 14, 2005. The positions will remain open until they are filled. *The University of Toledo is an Equal Access/Equal Opportunity/Affirmative Action Employer and Educator.*

ANNOUNCEMENTS

UNIVERSITY OF CAMBRIDGE
Science Summer School
17 July–6 August 2005

Study with some of Cambridge's finest scientists and other leading experts. Choose from a broad range of science topics to suit your particular interests. Further information at **website: http://www.cont-ed.cam.ac.uk/IntSummer/sschools/Science/. E-mail: intenq@cont-ed.cam.ac.uk. Telephone: +44 (0)1954 280398** (quote ref:SCI).

AAAS Annual Meeting and *Science* Career Fair

The 2005 AAAS Annual Meeting is the perfect place to explore your career options and attend the *Science* Career Fair. Both the career fair and the career-related workshops are FREE to attend.

AAAS Annual Meeting

DATES: **17–21 February 2005**

PLACE: **Marriott Wardman Park Hotel Washington, DC**

CAREER WORKSHOPS (see website for complete listing of workshops):

- Strategic Networking
- Pathways to Multiple Career Opportunities
- AAAS Fellowship Program in Public Policy and Mass Media
- Research Training at the NIH
- How to Fire Up your Presentation

AAAS/*Science* Career Fair

DATE: **21 February 2005**

PLACE: **Marriott Wardman Park Hotel Washington, DC**

TIME: **11:00 am – 4:00 pm**

Science Careers offers you the chance to meet employers.

Exhibiting employers are typically from biotechnology, pharmaceutical, government, and manufacturing organizations.

For more information and updates to our exhibitor list, please visit www.sciencecareers.org and click on Career Fairs.

▶ **Registration is required to attend the career workshops. Visit www.sciencecareers.org and click on Career Fairs for instructions on how to register for free.**

ScienceCareers.org

We know science AAAS

2005 FASEB Summer Research Conferences

Saxtons River, Vermont

- Autoimmunity
- Mechanisms & Regulation of Prokaryotic Transcription
- Nuclear Structure & Cancer – ***Co-sponsored by The Endocrine Society***
- Proteases in Hemostasis & Vascular Biology
- Transport ATPases: Genomics, Mechanisms, & Relevance to Diseases
- Perspectives in Transport Biology
- Hematological Malignancies
- New Insights in Polycystic Kidney Diseases: Molecular Pathways, Pathogenic Mechanisms, & Translational Applications

Tucson, Arizona

- Mammalian Mobile Elements
- Skeletal Muscle & Stem Cells
- Biology & Chemistry of Vision
- Intracellular RNA Sorting, Transport & Localization
- Lymphocytes & the Immune System: Molecular, Cellular & Integrative Mechanisms
- Calcium Oxalate in Biological Systems
- Immunoreceptors
- Nutrient Control of Gene Expression & Signaling **– *Co-sponsored by The Endocrine Society***
- Growth Factor Receptor Tyrosine Kinases in Mitogenesis, Morphogenesis & Tumorigenesis

Snowmass Village, Colorado

- Regulation of Ion Channel
- Lysophospholipid Mediators in Health and Disease
- Signal Transduction in the Immune System
- TGF-ß Superfamily: Signaling & Development
- Chromatin & Transcription
- Protein Kinases & Protein Phosphorylation
- Genetic Recombination & Genome Rearrangements
- Receptor & Signal Transduction **– *Co-sponsored by The Endocrine Society***
- Glucose Transporter Biology **– *Co-sponsored by The Endocrine Society***
- Gastrointestinal Tract XI: Innovations in GI Research & Therapy

Toscana, Italy

- Ciliate Molecular Biology

Mailing List
To be added to our mailing list, contact:
FASEB Summer Research Conferences
FAX: 301-634-7007
jlevin@faseb.org; mcgovern@faseb.org; shamilton@faseb.org

Information & Application
Available in March on our web site – http://src.faseb.org
FASEB Members will Automatically Receive the Meeting Notice

DNA Sequencing Services

SNP Discovery/Resequencing/ Variant Discovery	**$0.01 per base, per direction**
High throughput sequencing	**$5 per reaction**
cDNA sequencing	**$5 per reaction**
S.A.G.E. sequencing	**$5 per reaction**

Custom Genomic Services

Custom Gene synthesis	**$1 per base pair**
PCR	**$1 per amplicon**
Real Time PCR	**$500 per assay**
Whole genome scanning	**$5 per locus, per sample**
Nucleic acid extraction/ purification	**$2.50 per mini-prep**
Mutagenesis	**$400 any mutation within 5 bp frame**
Subcloning	**$400 per clone**
Library construction: Genomic, cDNA, BAC	**Please inquire for pricing**

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